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13. ABSTRACT *(Maximum 200 Words)*

Breast cancer mortality is higher among West African and African American women than it is among caucasian women in the United States, but the reasons for theses difference are not known. To devise effective methods for preventing breast cancer, we must understand which factors alone or in combination raise the risk of triggering a tumor, and which factors protect against the disease. Specific goals for the proposed research are to: (1) identify and validate the risk factors that can be modified to reduce breast cancer risk, and (2) achieve a better understanding of how various genetic and environmental factors interact to affect the risk of breast cancer. To reach these goals, we need a multidisciplinary training program and greater collaboration among investigators from diverse disciplines and institutions.

The goal of the current proposal is to obtain the necessary training needed to create an atmosphere at the Cancer Center at Howard University as a leading breast cancer training institute. It is also critical that multidisciplinary training take place so that individuals can participate effectively in multi-investigator collaborations that bring basic research discoveries to the bedside. Breast cancer research is increasingly becoming a multidisciplinary endeavor that requires a strong training program and better communication among investigators.

Appropriate animal models and models of human mammary cell and organ culture are urgently needed to accelerate progress in breast cancer research. This realm of research will require resources for necessary training, the development of animal models, technology development and access, and collaboration between Walter Reed Army Institute of Research and the Howard Cancer Center in diverse disciplines. Workshops in animal handling techniques by Division of Veterinary Medicine, Walter Reed Army Institute of Research will be provided for Howard investigators.

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Introduction

The peak incidence of breast cancer in African Americans and West Africans occurs around the premenopausal period while it occurs postmenopausal period in whites. Also, unlike white women, West-African and African-American women present late for treatment with a greater cancer burden and consequently lower survival rates. Breast cancer mortality is higher among African American women than among white women in the United States. The reasons for these racial differences are still not very clear, therefore we would aim to study some of these differences at the molecular and cellular level in these two different populations.

To devise effective methods for preventing breast cancer, we must understand which factors alone or in combination raise the risk of triggering a tumor, and which factors protect against the disease. Specific goals for the proposed research are to: (1) identify and validate the risk factors that can be modified to reduce breast cancer risk, and (2) achieve a better understanding of how various genetic and environmental factors interact to affect the risk of breast cancer. To reach these goals, we need a multidisciplinary training program and greater collaboration among investigators from diverse disciplines and institutions.

The goal of the current proposal is to obtain the necessary training needed to create an atmosphere at the Cancer Center at Howard University as a leading breast cancer training institute. Opportunities to acquire certain relevant skills are not readily available for HU faculty, postdoctoral staff and students at various stages in their training so a partnership has been established with WRAIR to fill this need.

There are several aspects of breast cancer research offered by Walter Reed Army Institute of Research that will be very important to our progress toward becoming a Breast Cancer Center at Howard University.

a) The first of these is a skill lacking in most laboratories addressing breast cancer; detailed pathological and biological studies of normal mammary glands at different stages of development using whole mouse mammary gland organ cultures. Dr. Barbara Vonderhaar developed this technique and she and Dr. Rina Das have used it for the past 5 years. The two of them will conduct training workshops utilizing those systems. In addition to the whole organ cultures, eventually we plan that we might use partial organ cultures of human tissue samples.
b) Differential display PCR, gene array technology, and other molecular biology methods have been used extensively in Dr. Jett's laboratory over the past several years. She and Dr. Das have become expert in the identification of genes differentially expressed using these systems. The techniques are used constantly in their laboratories and will provide an ideal setting in which to utilize these novel methods.
c) Use of animals in breast cancer research is frequently an important extension of testing ideas. The Howard University Cancer Center does not have an animal facility at the present time. Therefore, the extensive facilities and training offered by the Walter Reed Army Institute of Research will provide opportunities for investigators to participate in this important aspect of research. Dr. Jett has extensive experience writing animal use protocols and has used rodents, swine and non-human primates in her research and will advise in all aspects of animal use proposals. In addition, the staff in the Division of Veterinary Medicine will permit us to join classes designed to provide hands-on experience for learning procedures and understanding regulations in the use of any type of animal desired.

d) The main objective of this program is to provide training to all investigators at the Howard Cancer Center who would like to have individual experience in strategies for preparation and writing grant proposals.

Drs. Agnes Day and John Stubbs will be the lead faculty for this training at the Howard Cancer Center. The postdoctoral associates working with them, graduate students and other faculty may participate in this training process. Dr. Stubbs will focus his studies on the extracellular matrix involvement in breast cancer, Dr. Day will direct her efforts toward studies of regulation of metastasis and Drs. Das and Jett will focus on silencing of transcriptional genes related to breast cancer. These three efforts will eventually aim to utilize tissues from African-American women.

BODY

Key accomplishments and reportable outcomes: The DOD funded HBCU/MI Partnership Training award entitled "Breast Cancer in African American Women: Molecular Analysis of Differences in Incidence and Outcomes" has had a very auspicious beginning. Our initial goals for year 01 were two-fold:

- To attract pre-doctoral students, post-doctoral fellows and faculty to breast cancer research via introductory symposia, hands-on workshops, specialized training and research opportunities.
- To perform breast cancer research at both sites, Howard University Cancer Center (HUCC) and Walter Reed Army Institute for Research (WRAIR) which would add to the knowledge base and ultimately lead to the understanding, cure and/or prevention of this disease.

Towards these goals, we have accomplished the following:

Administrative:

- A Cooperative Research and Development Agreement (CRADA) has been established between Howard University and WRAIR for exchange of funds between the two institutions, with clearly defined SOWs for each Institution's participation.
- A full time Research Associate was hired at HUCC. A part-time administrator was hired at WRAIR to assist with the program coordination between sites.
- An advertisement for two full time postdoctoral positions has been created and the search continues. Various scientific journals and associations have been used to advertise for this position.
- Monthly meetings were implemented between the key investigators and coordinator at HUCC and WRAIR. These meetings are supplemented with e-mail and Tele-conferencing.
- An Access-based database has been created to track all individuals who are enrolled in this training program, including their areas of interest.
- HUCC has reorganized research labs around organ systems. Dr. Day is leader of the Breast Cancer Working Group. This allows for cross-fertilization of ideas and more interdisciplinary and collaborative research.

Introductory Symposia and Workshops:

- "Animal Models of Breast Cancer" Workshop – June 19, 2001. An overview of the goals and structure of the training program, introductory remarks from members of WRAIR and HU involved in the program, presentations from Deans of Research, Chairs of IACUC from HU and WRAIR and invited presentations by established cancer investigators from NCI, NIH and NIDDK, NIH and WRAIR. Presentation topics included animal models of breast cancer, mammary gland development, growth regulatory mechanisms of hormones during normal

mammary gland development and tumorigenesis, the use of experimental mouse genetics to understand mammary gland development, transgenic mouse models and In vivo Imaging of breast cancer in animals. Site of Performance-HUCC (105 participants).

A program book with abstracts and biographies of all speakers was created and disseminated to all participants.

- "Conducting Research Responsibly" Satellite Teleconference-September 13, 2001. Topics on human subjects research, conflict of interest, research misconduct and mentorship were discussed. Panel members included representatives from Office of Human Research Protection, NIH; American Association of Medical Colleges; Office of Research Compliance and Assurance, Veterans Administration; Office of Research Integrity, HHS and American Association for the Advancement of Science. Site of Performance-HUCC. (25 participants)

Hands-on Workshops

- "Rodent Handling and Techniques" Workshop-July-September, 2002. A total of 4 graduate students, 1 postdoctoral fellow and 3 faculty From Howard University have taken the 5-hour workshop offered at WRAIR. The workshop included lectures on various types of rodents used in research; and humane methodology and techniques used in injecting, bleeding and euthanising experimental animals. The lecture was followed by laboratory exercises in which each participant worked with mice and rats to perfect the various techniques described in the lecture. A waiting list for this workshop is currently at 8 HU individuals (students and faculty). WRAIR has offered to hold a workshop for HU individuals at a time of our choosing, since the class size is limited. Site of performance-WRAIR.
- Mammary Gland Dissection Workshop – September 2002. Hands-on laboratory procedures on dissection and staining of virgin, pregnant, lactating and involuting mammary gland to visualize normal mammary gland development. This workshop will be offered quarterly two HU faculty have participated. Site of Performance- WRAIR.

Newly Established Collaborative Research Projects

The projects listed below are in the formative phase and will be implemented in year 02.

- Dr. Rina Das (WRAIR) – Dr. Aleayehu Kassa (HUCC) Selective Estrogen Receptor Modulators and Depression in a Mouse Model of Breast Cancer. This is a recently established collaboration that is still in the planning phase. Dr. Kassa has taken the rodent handling and techniques workshop and is currently preparing a mini proposal on the use of WRAIR's animal models and in vivo imaging for this project.
- Dr. Rina Das (WRAIR) – Dr. John Stubbs (HUCC) Use of inducible vectors along with luciferase vectors to study the effect of bone sialoproteins on metastasis of breast cancer in animal models.
- Dr. Marti Jett (WRAIR) – Dr. Agnes Day (HUCC) Microarray analysis of selected genes in normal and cancerous cell lines grown on plastic-vs- matrigel.
- Dr. Rasha Hammamieh (WRAIR) – Dr. Robert Canada (HUCC) Effects of anti-cancer agents on normal and cancerous breast organ cultures.

Dr. Day's research progress: Research has been focussed on the expression of connective tissue protein genes in normal, transformed, primary cancerous and metastatic cancer cells. Initial studies have shown differential expression of these genes within cancer types (breast); between cancer types (breast-vs-colon) and between *in situ* sites (solid tumors-vs- ascites or pleural effusion. (See Appendix: Era of Hope Abstract, 2002). These experiments will be repeated after growing cells on

a basement membrane substrate (matrigel). Comparisons will then be performed between clinical samples from African American patients and the cell lines currently in use. Microarray analysis of gene expression in current cell lines has begun, and will also be compared to clinical samples, when available.

Students Trained:

All of the graduate students listed below have been supported-in-part by this training program. Most have participated in all of the workshops and all are involved in research projects involving cancer.

- Elizabeth Nelson, 3rd year graduate student (Microbiology). Gene regulation in Breast Cancer.
- Tamara Tatum-Broughton, 4th year graduate student (Microbiology). Gene expression in cervical and ovarian cancer.
- Douglas F. White, 2nd year graduate student (Microbiology). Molecular analysis of skin cancer and heritable diseases.
- Aliesha Dobbins, 4th year graduate student (Biochemistry). Enhancement of SP6 Polymerase production for molecular biological use.
- Gay Morris, 5th year graduate student (Biology). Molecular analysis of lymphatic and breast cancer.

Grants Submitted

- Agnes A. Day. Molecular and Microarray Analyses of Connective Tissue Protein Gene Expression in African American, Caucasian and Korean Breast Cancer Samples. Howard / Hopkins Partnership Pilot Project Initiative. $49,800. Dec.2002-Nov.2003 (Pending).
- John T. Stubbs. Effects of extracellular matrix proteins on prostate cancer cells. NIGMS/NIH, $461,000; 2002-2006. Funded.

Promotions, Honors and Awards:

- Agnes A. Day and John t. Stubbs: American Association for Cancer Research, HBCU faculty Scholar Travel Award to attend the "metalloproteases, Extracellular Matrix and Cancer" meeting, Oct 9-11, 2002. Hilton Head, SC.
- Agnes A. Day. Interviewee, "Will We Win The War Against Microbes?" episode of Closer To Truth Television Series. Los Angeles, CA, March 2001. To be aired on PBS and WHUT in late fall.
- Agnes A. Day. Associate Director for Basic Research, HUCC. March 2001-Present.
- Rasha Hammamieh. Scientist at WRAIR. American Society for Biochemistry and Molecular Biology, Scholar Travel Award and support to attend the Experimental Biology Meeting, April 2002 in New Orleans, LA.
- Rasha Hammamieh. Scientist at WRAIR. DOD Era of Hope Breast Cancer Research Symposium. Sept. 25-28, 2002. Orlando, FL. Full award to support travel, registration and per diem for the duration of the meeting.

Presentations

- Yancy, Haile, Nelson, Elizabeth, White, Douglas, Tatum-Broughton, Tamara, George, Matthew and Day, Agnes. 2002. Molecular analysis of connective tissue Protein Gene Expression in Various skin Diseases. American Society for Biochemistry and Molecular Biology, LB162: 33. New Orleans, LA

- Day, Agnes, Nelson, Elizabeth and George, Matthew. 2002 Molecular profiling of connective tissue protein gene expression in breast and colon cancer cells. DOD Era of Hope Breast Cancer Research Symposium. Sept. 25-28, 2002. Orlando, FL
- Hammamieh, R., R. Thomas, R. Das, and M. Jett. Anti-sense oligodeoxynucleotide complementary to liver fatty acid binding protein alters cellular functions in breast cancer cells. DOD Era of Hope Breast Cancer Research Symposium. Sept. 25-28, 2002. Orlando, FL
- Hammamieh, R., K. Carr, C. Dulaney, R. Das, and M. Jett. Study of the effect of omega-3 and omega-6 fatty acid on cell growth and oxidative stress in breast cancer cells. DOD Era of Hope Breast Cancer Research Symposium. Sept. 25-28, 2002. Orlando, FL

Courses:

- Oncology. This is a multidisciplinary graduate level course, which will be offered through the Microbiology department. Topics include cancer biology, genetics of cancer, viral vectors in cancer, oncogenes, hormonal regulation of cancer, and cancer epidemiology. Lecturers will be from Howard University, Johns Hopkins University, Georgetown University, Walter Reed Army Institute for Research, and the National Cancer Institute. The course is designed for 2 semesters, 4 credit hours each. It will be open to undergraduates, graduates, postdoctoral fellows, medical students, medical residents and faculty.
- Molecular Biology. The current molecular biology course offered within the Department of Microbiology will be enhanced through the addition of didactic lectures and/or laboratories on the following subjects: Gene array analysis; real time PCR; DNA methylation analysis; prokaryotic and eukaryotic expression vectors; stable eukaryotic transformation and selection; and phage display analysis of protein-protein interactions.

Tissue Repository: This facility has been established in the HUCC and will provide a source of cancerous tissue from breast, colon, prostate and other organs of African Americans and other ethnic groups. An individual with a strong background in cell and molecular biology and biotechnology is being hired to coordinate this laboratory. The HUCC Director's financial support of the Laboratory Coordinator and the dedication of two laboratories for the preparation and storage of these tissues evidence the commitment to this training program.

Conclusions

Excellent progress has been made towards fulfilling the specific aims outlined in the first year's statement of work. To fully utilize the training opportunities, offered by this program, in a logical progression, some of the goals designated for year 02 were re-scheduled to year 01, and vice versa. We had to enroll participants into the Animal Handling course first as per WRAIR requirement, which will then allow them to carry out other animal related research and training activities at WRAIR. We envision year two being equally productive in recruiting faculty and students for participation in other planned workshops, which will consist of new topics for established trainees (in vivo imaging, SCID mice with xenografts, Biostatistics and experimental design), and a continuation of the animal handling and mammary gland workshops and collaborative research projects. Year 02 will focus on basic research performance within the established collaborations augmented by animal use protocol preparation, grant writing workshops, and research proposal submissions for external funding.

APPENDIX

Documentation of accomplishments:

i. Publications and presentations
ii. Certifications
iii. Workshops
iv. Collaborative Research Projects
v. Oncology course syllabus
vi. Funding applications submitted
vii. Database for tracking training/collaboration outcomes

Experimental Biology

2002

"Translating the Genome"

New Orleans, LA

Late-Breaking Abstracts

various chemotherapeutic drugs. Using the 041 cells as our negative control, FACS analysis was conducted on samples with no induced DNA damage as well as samples treated with Camptothecin (CPT), Mitomycin C (MC) and Zeocin (Zeo) for the time points of 4 hrs., 24 hrs., and 48 hrs. TR9-7 (-tet) samples showed an overall increased level of apoptosis with and without DNA damage. However, the changes in apoptosis ad G1 arrest induced by the addition of MC and CPT were the same for both cell lines. Zeocin causes a significant G2 in the absence of p53 and did not cause G2 arrest in TR9-7 cells with induced p53. This preliminary data show that these drugs can cause apoptosis as well as growth arrest independent of p53.

LB160

cDNA Library Construction and Library Transfer Using Gateway Technology

Barry Robert Neilditch[1], Jon D Chesnut[2], Mark R Smith[2], Larissa Karnaoukhova[2], Chris E Gruber[2]. [1]R&D, Invitrogen Inc, 1600 Faraday Ave, Carlsbad, CA 92008, [2]Invitrogen Inc, Carlsbad, CA

Invitrogen's GatewayTM Technology is a versatile and efficient system for cloning DNA based on a lambda phage recombinase that can be applied in vitro to stimulate site specific (att) recombination. One application of this technology is the construction of robust directional cDNA libraries.1 Unlike standard cDNA cloning protocols, this approach does not require restriction enzyme digestion of the cDNA to create unique ends suitable for directional cloning. Construction of a library in an Entry vector allows for efficient transfer into a variety of Destination vectors creating an expression-ready library without compromising library complexity . Primary libraries constructed with this method routinely consist of >10e7 cfu and average insert sizes of > 1.5 kb. GatewayTM cloning combined with the GeneRacerTM technology has also been applied to clone cap-selected cDNA and create full-length enriched cDNA libraries.

1. Ohara,O. and Temple, G. (2001) Nucleic Acids Research 29 (4), E22.

LB161

Goat Uromodulin Promoter Drives GFP Kidney-Specific Expression in Transgenic Mice

Yue-Jin Huang, Nathalie Chretien, Annie Bilodeau, Jiang Feng Zhou, Anthoula Lazaris, Costas N Karatzas, Nexia Biotechnologies Inc., 1000, Ave. St-Charles, Vaudreuil-Dorion, Quebec J7V 8P5 Canada

Uromodulin is the most abundant protein in the urine of mammals. In an effort to use uromodulin promoter to target rc-proteins in the urine of transgenic animals we have cloned a 1.5 kb fragment of the goat uromodulin (GUM) and localized the gene by FISH analysis to chromosome 25. GUM promoter-GFP cassettes were constructed and transgenic mice were generated in order to study the promoter's tissue specificity and GFP kidney distribution. Tissues collected from three GUM-GFP transgenic lines, and analyzed by Western blotting and fluorescence confirmed that the GUM promoter drove expression of GFP specifically in the kidney. Using immuno-histochemistry analysis of kidney sections, GFP expression was co-localized, with endogenous uromodulin protein, in the epithelial cells of the thick ascending limbs of Henle¡¯s loop (TAL) and the early distal convoluted tubule in the kidney. These results illustrate that the GUM promoter may be a useful tool to target proteins in the kidney.

LB162

Molecular analysis of connective tissue protein gene expression in various skin diseases.

Hailé F. Yancy[1], Elizabeth Nelson[2], Douglas White[2], Matthew George, Jr.[3], Agnes A. Day[4]. [1]Cancer Center, Howard University, 2041 Georgia Ave.,NW, Washington, DC 20060, [2]Microbiology, Howard University, Washington, DC, [3]Biochemistry & Molecular Biology, Howard University Cancer Center, Howard University, Washingon, D.C., District of Columbia, [4]Microbiology, Cancer Center, Howard University, Washington, DC

Research has shown a direct correlation between genetic defects in collagen and elastin in skin diseases such as cutis laxa (CL), pseudoxanthoma elasticum (PXE), and Ehlers-Danlos syndrome (EDS). However, the genetic status of other connective tissue proteins (CTPs) has not been ascertained. The goal of this study was to assess the genetic status of type I collagen, decorin, osteonectin, and fibronectin in the diseases PXE, CL, EDS, xeroderma pigmentosum (XP)and malignant melanoma (MM).Total RNA, genomic DNA, and proteins were extracted from each cell line. Slot blot and RT-PCR analyses determined the transcriptional status of each CTP gene. Restriction fragment length polymorphism (RFLP), single strand conformation polymorphism (SSCP), and protein truncation test (PTT) analyses of the cell lines were performed to detect possible mutations.Two dimensional gel electrophoresis was performed to analyze the protein fingerprints of the diseases. Results indicate differential transcription occurred among pathologic cell lines. RFLP and SSCP analysis demonstrated altered gene structure of several CTP encoding genes. The PTT for decorin exhibited no mutations leading to a truncated protein. The 2-D protein fingerprint of MM and XP were different from normal skin. These results indicate there may be additional genetic defects contributing to the etiology of these diseases. Research support:DOD- BCRP grant DAMD 170110268; NIH, NCI grant RO3CA68991.

LB163

Investigation of the Kinetics of DNA Unwinding by Helicase I: Optimization of Oligonucleotide Substrates and Reaction Conditions

Bartek T Sikora[1], Alan J Tackett[1], Steven W Matson[2], Kevin D. Raney[1]. [1]Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, 4301 W. Markham, Little Rock, AR 72205, [2]Biology and Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC

Helicase I is a multi-functional enzyme required for DNA transfer in bacterial conjugation. The enzyme is encoded by the *traI* gene of the *Escherichia coli* F factor. The enzyme exhibits 5'-to-3' helicase activity and is classified in helicase superfamily 1. In order to characterize the helicase activity, conditions and oligonucleotide substrates were optimized. DNA fork substrates were designed containing a 5' and 3' ssDNA tail adjacent to a 30 base pair duplex. The 3'-ssDNA tail was 30 nt whereas the length of the 5'-ssDNA tail was varied. Unwinding of the substrate containing a 5'-60 nt ssDNA tail was faster than substrates containing 45 or 30 nt. Unwinding of the optimal substrate was measured under conditions of excess enzyme at 37 °C by using a Kintek rapid quench-flow instrument. The resulting pseudo-first order rate constant of 42.4 s^{-1} corresponds to 1,270 base pairs s^{-1} for the 30 base pair substrate, making Helicase I one of the fastest helicases studied to date *in vitro*.

LB164

***In vitro* mutant mixing studies of NS3 helicase of HCV**

bhuvanesh dave[1], Alan J Tackett[2], Kevin D. Raney[2]. [1]Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, 4301 West Markham, Slot 516, Little Rock, Arkansas 72205, [2]Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR

The oligomeric state of NS3, a helicase of Hepatitis C virus has been a controversial issue in the helicase field for sometime. It has been postulated to be a monomer based on X-ray crystallography, a dimer based on gel filtration assays, and an oligomer based on protein- protein cross linking assays. In order to resolve the issue, an ATPase deficient mutant was made by point mutation (DECH-AECH) in helicase domain II. This was purified using chromatographic techniques. A spectrophotometric ATPase assay was used to confirm lack of ATPase activity in the mutant. The binding constant (KD), determined by fluoresence polarization, was similar to the wild type NS3 protein. Furthermore, mutant NS3 did not unwind a 45-30 mer DNA substrate) under both excess enzyme and steady state conditions. These observations confirm that the mutant is ATPase deficient and lacks unwinding activity. Wild type NS3 protein has been shown to unwind DNA under steady state conditions. The dominant negative effect of mutant NS3 will be tested by mixing it with wild type NS3 under steady state conditions.

PROTEIN STRUCTURE AND FUNCTION

LB165

The SCAN Dimerization Domain Defines a Novel Family of Vertebrate-Specific Zinc Finger Transcription Factors

Tara L Sander[1], James R Stone[2], Jenny L Maki[1], Stephen C Blacklow[3], Tucker Collins[1]. [1]Department of Pathology, Children's Hospital, Harvard Medical School, 300 Longwood Ave., Boston, MA 02115, [2]Departments of Pathology, Brigham and Women's Hospital and Children's Hospital, [3]Department of Pathology, Brigham and Women's Hospital

The SCAN domain is a conserved protein interaction motif found predominantly in C_2H_2 zinc finger proteins of the *Krüppel*-type. From an extensive search of Celera and NCBI databases, we identified sixty-one genes in the human genome that contain a SCAN domain. Lower eukaryotes such as the worm and fly do not appear to contain SCAN genes, suggesting that the SCAN family is vertebrate-specific and has undergone significant lineage-specific expansion during recent evolution. The genes for SCAN family members are distributed throughout the human genome, with clusters

Molecular Analysis of Connective Tissue Protein Gene Expression in Various Skin Diseases

Haile F. Yancy,[1] Elizabeth E. Nelson[2], Douglas F. White[2], Tamara A. Tatum-Broughton[2], Matthew George Jr[3], and Agnes A. Day[2]. Department of Biology[1], Department of Microbiology[2], and Department of Biochemistry[3], College of Medicine Howard University, Washington, D.C. 20059.

Abstract

The goal of this study was to assess the genetic status of type I collagen, decorin, osteonectin, and fibronectin in the diseases Pseudoxanthoma elasticum, Cutis laxa (CL), Ehlers-Danlos(ED), Xeroderma pigmentosum (XP), and Malignant melanoma (MM). These diseases, with the exception of Ehlers-Danlos, have not been previously shown to result from connective tissue proteins (CTP) defects. Total RNA, genomic DNA, and proteins were extracted from each cell line. Slot blot and RT-PCR analyses determined transcriptional status of each CTP gene. Restriction fragment length polymorphism (RFLP), single strand conformation polymorphism analysis (SSCP), and a protein truncation test (PTT) analysis of the cell lines were performed to detect possible mutations in the genes of interest. RFLP and SSCP analyses demonstrated altered gene structure of several CTP encoding genes. The PTT of decorin exhibited no mutations leading to a truncated protein. These preliminary results indicate that there may be genetic defects, other that those currently cited, contributing to the etiology of these diseases.

Introduction

Xeroderma pigmentosum (XP) is a rare pigmentary and atrophic disease. It is an autosomal recessive inherited familial disease with an altered reaction of the epidermis to light (1). The disease is characterized by the skin's sensitivity to light of wavelength 780 - 340 nm. XP normally starts in childhood and often leads to keratoses, papillomas, carcinomas and melanomas. A loss of vision is often associated with the disease. Although the mild forms of the disease give no rise to any serious problems, if left untreated death can occur in early adulthood from the metastasis of the malignant lesions (2).

Pseudoxanthoma elasticum (PXE) is a disease that affects the skin, eye, and the cardiovascular system. The skin of patients with PXE is usually characterized by small, circumscribed, yellowish to cream colored patches at sites of considerable movement of the skin such as the sides of the neck (3). Gradual loss of vision often occurs after minor trauma to the eye, such as scratches on the cornea. Patients often suffer from diabetes mellitus, calcinosis, and osteitis deformans (4).

Ehlers-Danlos (ED), also known as Cutis Hyperelastica, is a group of inherited disorders which affect dermal connective tissue to produce symptoms of hyper-elasticity of the skin, abnormal scar formation, bruising, skin calcification, and joint hypermobility. Eleven types of ED result from defects in the interactions and stability between collagen molecules and abnormality in collagen fibrillogenesis (5). Any disruption in the organization of collagen fibrils would insult the integrity of the skin.

Malignant Melanoma (lentigo maligna) begins as a tan macule that extends peripherally, with gradual uneven darkening over the course of several years. The spread and darkening are usually so gradual that little attention is paid to this insidious lesion. Its edges become irregular with time, and its color variegated, due to areas of regression. After a radial growth period of 5 to 20 years vertically growing melanoma usually develops within the lesion (6). Green has shown that a combination of light complexion, light eyes, and blond or red hair indicate increased risk for melanoma, given comparable sunlight exposure (7). Other risk factors include the presence of precursor lesions such as congenital and dysplastic nevi, the occasional development of melanoma in large burn scars and xeroderma pigmentosum (8).

There have been numerous reports on the reduced levels of type I collagen and elastin in PXE (9) and ED (10). The expression of fibronectin has been studied in PXE, but to date there are no molecular studies published that involve the analysis of the connective tissue proteins fibronectin, osteonectin, and decorin-which may also play a role in the clinical aspects of these diseases. Day et al have demonstrated that several connective tissue proteins, i.e. type I collagen, decorin, and osteonectin are under coordinated regulation (11). Thus, if the transcriptional level (mRNA production) of one of these proteins is altered in a disease, then there is a high probability that the other connective tissue proteins will also undergo altered expression.

The present study was designed to demonstrate the presence of abnormal transcription and mutations of decorin, type I collagen, fibronectin, and osteonectin in these diseases. Analysis of transcription was accomplished by slot blot and RT-PCR analysis. Mutations of theses genes were analyzed by restriction fragment length polymorphism analyses (RFLP), single strand conformation polymorphism analyses (SSCP), DNA sequencing, and protein truncation tests (PTT).

Materials and Methods

Cell culture

Normal human dermal fibroblasts (N), dermal fibroblast cells affected by Xeroderma pigmentosum (XP), Pseudoxanthoma elasticum (PXE), Ehlers-Danlos (ED), and Malignant Melanoma (MM) were purchased from American Type Culture Collection (Rockville, MD, USA). The N cell line was cultured in 90% Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum. XP, PXE, MM, and ED were cultured in 90% Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum. The cells were placed in a 5% CO_2 humidified incubator. Cells were propagated in the recommended media and given new media every 2 to 3 days for 2 to 3 weeks.

RNA and DNA Extractions

Total RNA was isolated from the cell lines as outlined by the RNAzol B preparation kit (Tel-Test, Friendswood, TX). This solution is a modification of the method of Chomczynski and Sacchi. DNA was isolated from the cell lines outlined by the Stratgene extraction kit (Stratgene Lajolla ,CA). The solutions used were based on the genomic DNA isolation method from tissue culture cells reported by S.A. Miller, et.al,. Both RNA and DNA samples were standardized to a final concentration of 1.0 ug/ul.

Slot Blot Analysis

Uniform aliquots of total RNA (0.25µg, 0.5µg, 1.0 µg) were denatured using 20X SSC, and 37% formaldehyde and heated at 60°C for 15 minutes. The samples were spotted onto nitrocellulose membrane strips using Scotlab Slot Blotter (Scotlab, Ltd. Strathclyde, Scotland) apparatus under vacuum using a pressure a 100 mm Hg. The strips were labeled and baked for two hours at 70°C . The blots were prehybridized for 10 hours at 37°C in hybridization solution (50% formamide, 6X SSC, 5X Denharts, 0.1% SDS, and 100 ug/ul denatured fragmented salmon sperm DNA). The membranes were then singly hybridized with the ^{32}P-labeled cDNA inserts corresponding to the CTPs (containing 3.0 x 10^7 cpm/ml) overnight in a 37°C water bath. The next day the membranes were washed once with 2X SSC/ 0.5% SDS for 5 min. at RT, 2X SSC/ 0.1% SDS for 15 min. at RT, 0.1% SSC/ 0.5% SDS for 5 min. at 37°C, 0.1X SSC/ 0.5% SDS for 45 min. at 68°C, and 0.1X SSC for 5 min. at RT. The membranes were autoradiographed for 1 to 3 days. The autoradiograms were then subjected to densitometric measurements using a Chemi Imager Tm 4000, (Alpha Innotect Corporation San Leandro, CA) to quantitate the transcription of the RNA for each probe.

Restriction Fragment Length Polymorphism (RFLP) Analysis of Genomic DNA

Genomic DNA (10µg) was digested with restriction endonucleases under conditions recommended by the manufacturers (Life Technologies, Inc. Gaithersburg, MD: Promega Corp., Madison, WI) for 2 to 4 hours. The restriction enzymes used in this study include Bam H I, Hind III, and Kpn I. The digested DNA was electrophoresed on a 0.8% agarose gel for 2 to 4 hours. The gels were stained with 0.5ug/ml of ethidium bromide for 20 min. and photographed.

The agarose gels were then depurinated with 0.25mM HCl. Following depurination, the gels were then rinsed with dH_2O for 5 min. The agarose gels were then washed twice, for 30 minutes, with Solution 1 (0.5M NaOH, and 1.5M NaCl) to denature the DNA and neutralized in two 30 min. washes with Solution 2 (9.5M Tris, pH 7.5, and 3.0M NaCl). Capillary transfer for 12 hours transferred the neutralized DNA from the agarose gels to nylon membranes. After the 12-hour transfer, the nylon membranes were removed and baked for 2 hours at 70°C. The nylon membranes were then hybridized as stated above.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The RT-PCR reactions were performed in a GTC-2 thermocycler (GL Applied Research), using the Access RT-PCR system (Promega, Madison WI). The reaction mix was prepared by combining 27.5 µl of nuclease-free water, 10ul of AMV/Tfl 5X reaction buffer, 2µl dNTP mix, 1.5µl of upstream primer, 1.5µl of downstream primer, 3.0ul 25 mM $MgSo_4$, 1.0ul of AMV reverse transcriptase, 1.0ul of Tfl DNA polymerase and 3.0µl of total RNA, in a 0.50ml thin walled tube on ice. The reaction mix was then vortexed for 10 seconds to mix. The temperature cycling consisted of denaturing at 48°C for 1 minute for reverse transcription of the RNA into cDNA, 94°C for 4 minutes to deactivate reverse transcriptase, and 30 cycling sequences of denaturing at 94°C for 45 s, annealing at 45°C for 30 s, and extension at 72°C for 1 minute with a final extension at 72°C for 10 minutes. Twenty microliters of each RT-PCR reaction was electrophoresed on 1.2% agarose gels. Gels were stained with ethidium bromide and photographed using a Chemi Imager Tm 4000,(Alpha Innotect Corporation San, Leandro, CA).

Single Stranded Conformational Polymorphism (SSCP) Analyses

Genomic DNA was amplified with primers specific for the 1A1 promoter of decorin in the presence of ^{32}P-labeled dCTP. The denatured ^{32}P-labeled products were resolved by electrophoresis on non-denaturing 0.5 x MDE (FMC) gels with and without glycerol. The dried gels were exposed to film for 6-12 hours.

Protein Truncation Test (PTT)

Exons 1-8 of decorin were screened using reverse transcribed cDNA in the protein truncation test. cDNA was amplified with four sets of primers that spanned the eight exons of the decorin gene. Since the forward primer contained a promoter for T7 RNA polymerase, the corresponding protein was synthesized in vitro with a biotin label. The proteins were separated by sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis and exposed to film for 5 to 15 minutes.

DNA Sequencing

Genomic DNA was amplified and sequenced with both forward and reverse primers using fluorescent dye terminator cycle sequencing. Products were analyzed by an automated sequencer. In order to eliminate false readings due to Taq errors, two independent samples were sequenced, each sample contained 3 or more independent PCR reactions that were combined. Sequences were analyzed with the sequencer program from Gene Codes.

Results

Table 1

1828	Normal Skin	HTB 68	Malignant Melanoma
1194	Ehlers-Danlos/Pseudoxanthoma elasticum	1184	Pseudoxanthoma elasticum
1202	Xeroderma pigmentosum	1396	Cutis laxa

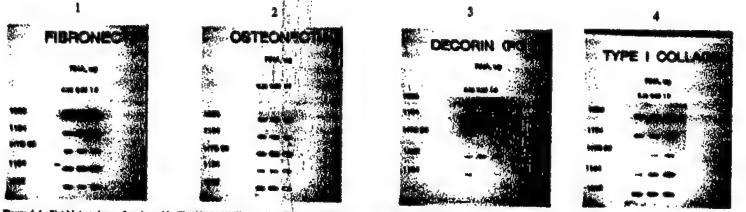

Figure 1-4. Slot blot analyses of various skin fibroblasts, (1) fibronectin, (2) osteonectin, (3) decorin, (4) type I collagen. Standardized amounts (0.25 µg, 0.50 µg, 1.00 µg) of total skin fibroblasts RNA were denatured in 20 X SSC and 37% formaldehyde and loaded onto nitrous Bolton filter membrane mounted within the sample well platform of the Scotlab slot Blotter. The membranes was baked and hybridized with ^{32}P-radiolabeled cDNA encoding the CTPs.

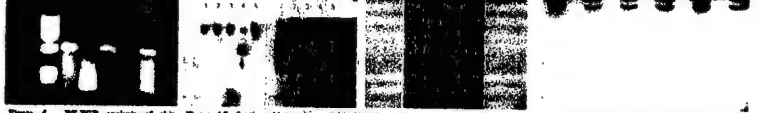

Figure 5. RT-PCR analysis of skin fibroblast RNA. 0.25 µg of total RNA was reversed transcribed and amplified for the decorin exons (PG 2F). The products were electrophoresed in a 1.5% agarose gel stained with ethidium bromide. Lane 1, marker; 2, 1828 (N); 3, 88 (LMG); 4, 1194 (ED/PXE); 5, 1184 (XP); 6, 1396 (CL).

Figure 6-7. Southern blot analyses of skin fibroblast DNA. Ten micrograms of genomic DNA digested with BamH I, Hind III, or Kpn I, were electrophoresed in 0.8% agarose gel stained with ethidium bromide. Separated DNA fragments were blotted onto nylon membrane, hybridized ^{32}P-labeled cDNA encoding type I collagen (6) lanes 1, (N); lanes 2, (LMG); lanes 3 (ED/PXE); lanes 4, (XP); lanes 5, (CL); and decorin (7) lane, (N); lane 2, (LMG); lane 3, (ED/PXE); lane 4, (XP); and autoradiographed.

Figure 8. SSCP analysis of decorin 1A1 promoter region. The SSCP results indicate that there are no detectable sequences differences in the cell lines1828 (N), lane 2; 88 (LMG), 3; 1184 (PXE/ED),4; and 1194 (XP), 6. In contrast to the prior diseases, 1194 (ED/PXE), 1 and 1396 (CL), 5 revealed a different electrophoretic pattern.

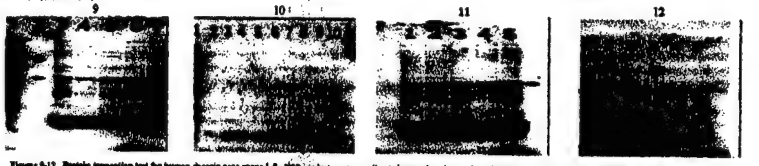

Figure 9-12. Protein truncation test for human decorin gene exons 1-8. PCR products corresponding to human decorin protein region exons 1A-3 (figure 9) exons 3-5 (figure 10), exons 4-7 (figure 11), and exons 7-8 (figure 11) were transcribed and translated. All decorin cell lines exhibited normal length decorin protein.

Summary

1. 1202 (XP) displayed a decreased level of expression of fibronectin, type I collagen, and decorin, but an equal level of expression of osteonectin when compared to the amount of expression of the CTPs in normal skin fibroblasts.

2. 1184 (PXE) displayed a decreased level of expression of fibronectin and type I collagen. It also had an increased level of expression of osteonectin and decorin ; when compared to the expression of the CTPs in normal skin fibroblasts.

3. 1194 (ED/PXE) displayed a decreased level of expression of fibronectin, type I collagen and decorin, but an equal level of expression of osteonectin when compared to the amount of expression of the CTPs in normal skin fibroblasts.

4. HTB-68 (MM) exhibited a decreased level of expression of fibronectin, type I collagen, and decorin. An increased level of osteonectin was observed when compared to the amount of expression of the CTPs in normal skin fibroblasts.

5. RFLP analyses indicated that there may be a mutation within the generated fragments of the type I collagen and decorin genes of PXE and ED when digested with BamH I, Hind III, and Kpn I.

6. SSCP analyses revealed mutations in the decorin 1A1 promoter sequence of ED/PXE (1194) and CL (1396).

7. The PTT analysis indicated that there are no mutations of any of the skin diseases that lead to a truncated decorin protein.

Discussion

Pseudoxanthoma elasticum is a complex inherited disorder that profoundly affects elastic tissue. The precise cause of the disease has not yet been determined, but the irregularly arranged or twisted collagen fibrils, calcification of elastic tissue, and the increased level of fibronectin (12) have been suggested as possible causes. Xeroderma pigmentosum, an extremely rare autosomal recessive disease is a result of a faulty DNA repair system (13). The general cause of Ehlers-Danlos is a defect in the interactions and stability between collagen molecules. Malignant melanoma is often seen in those who have risk factors that include dysplastic nevi, Xeroderma pigmentosum, (1), and a light complexion (2). The chief site for metastatic melanoma is the skin, but all other organs are at risk.

The characteristics and possible causes of the diseases have been well reported (14,15). The effect of abnormal CTPs in relation to these diseases is still unclear. The loss of fibronectin (figure 1) and osteonectin (figure 2) and their relationship to the pathology is not yet fully understood. The reduction of the expression of type I collagen (figure 4) could be attributed to the change in the gene encoding for the protein. The change in the banding pattern (figure 6) suggests that there could be a mutation within the regenerated fragments of the gene. This mutation could have occurred in the regulatory region of the gene which could reduce or eliminate the production of the protein. An example of this type of mutation was seen in the 1A1 portion of the decorin promoter (figure 8). This mutation could play a major role in the alteration of transcription of the decorin protein. The data also indicates that none of the diseased cell lines exhibited a mutation in the exonic region of the decorin gene leading to a truncated mutation. Based on the data presented it is not surprising that a large number of pathological conditions that have been described here may be directly attributed to abnormal connective tissue gene expression.

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Proceedings
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MOLECULAR PROFILING OF CONNECTIVE TISSUE PROTEIN SYNTHESIS IN BREAST CANCER CELLS

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The progression of normal cells to the benign, malignant and metastatic phenotype involves a highly complex series of events. Proteolytic degradation is the currently accepted method by which metastatic cells abrogate the basement membrane and connective tissue matrix to gain access to the circulatory and lymphatic systems for dispersal. Our studies were designed to ascertain whether there are alterations in the transcription of proteins of the extracellular matrix (ECM) which are concomitant with reported increased metalloprotease production, when metastatic cells are compared to their normal tissue counterparts, and when breast cancer tissue is compared between African American and Caucasian women.

Comparisons were made between paired sets of tissues or cells for the presence and / or quantitative levels of various connective tissue proteins and regulatory status of their promoters. Steady state levels and sizes of mRNAs were measured by hybridizing Northern blots and slot blots with radioactively labeled cDNA probes encoding decorin, fibronectin, osteonectin, and type I collagen. Southern blot analyses were done employing standardized concentrations of genomic DNA isolated from various breast cancer cell lines and the aforementioned probes.

Data from these studies show that there is differential expression of connective tissue proteins between various transformed cell lines. When comparisons of transcriptional status and genomic mutations were done between breast and colon cells, differential expression was also observed. Mobility shift patterns were generated by nuclear extracts from normal and cancerous breast and colon fibroblasts complexing with the fibronectin promoter. Alterations in the complexes interacting with sequences from the promoter may be responsible for the differences in fibronectin gene expression among normal and cancerous breast and colon fibroblasts. Collectively, these differences may play an important role in elucidating the metastatic phenotype of cancer. This research will provide a better understanding of the molecular events of cancer metastasis and, eventually, to the inhibition of this phenomenon.

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Molecular Profiling of Connective Tissue Protein Gene Expression in Breast and Colon Cancer Cells

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Abstract

The following studies were designed to ascertain whether there are alterations in the transcription of proteins of the extracellular matrix (ECM) which are concomitant with reported increased metalloprotease production, when breast cancer cells are compared to their normal tissue counterparts, and to colon cancer cells. Comparisons were made between sets of tissues or cells for the presence and/or quantitative levels of various connective tissue proteins. Steady state levels of mRNAs were measured by hybridizing slot blots with radioactively labeled cDNA probes encoding decorin, fibronectin, osteonectin and type I collagen. Restriction Fragment Length Polymorphism (RFLP) analyses were done employing standardized concentrations of genomic DNA isolated from various breast cancer and breast cell lines and the aforementioned probes. Data from these studies show that there is differential expression of connective tissue protein (CTP) genes among several types of breast cancer cell lines and between breast and colon cells.

Introduction

The progression of normal cells to the benign, malignant and metastatic phenotypes involves a highly complex series of events. Proteolytic degradation is the currently accepted method by which metastatic cells abrogate the basement membrane and matrix to gain access to the circulatory and lymphatic systems for dispersal. To metastasize successfully, cancer cells must detach from the primary tumor, invade local blood and/or lymphatic vessels, enter the circulation to travel to a distal site, extravasate and arrest at a vascular bed and proliferate as a secondary colony (1,2).

Reports in the literature have documented the increased synthesis of connective tissue proteins and the involvement of stromal components in various tumors (3, 4). Abnormal connective tissue matrix production termed the desmoplastic response, often surrounds neoplasms. This response involves the excessive production of matrix around groups of tumor cells . This response is most often associated with invasive tumors, diffuse infiltrative gastric carcinomas and infiltrating ductal carcinomas of the breast (5) Barsky et al. have postulated that this response is the host's protective reaction against tumor invasiveness and metastasis. The above cited studies indicate that while proteolytic digestion of the ECM is a strongly documented method of metastatic cells gaining egress from a primary tumor, there are also cellular mechanisms that play a role in both enhancing and retarding the movement of these cells. This study was designed to determine whether the genes encoding decorin, fibronectin, osteonectin and type I collagen undergo positive or negative regulation in a predictive manner during cancer induction and metastasis.

Materials and Methods

Cell Culture

All breast cell lines, (CRL 1828, HS578T, MCF7 and MCF10A) were obtained from American Type Culture Collection (ATOC, Manassas, VA) except A1N4, which was a gift from Dr. David Salomon, National Cancer Institute, NIH, Bethesda, MD. Culture media and supplements are detailed in Table 1. The cells were placed in a 5% CO_2 humidified incubator. Cells were propagated in the recommended media and given new media every 2 to 3 days for 2 to 3 weeks.

RNA and DNA Extractions

Total RNA was isolated from the cell lines as outlined by the RNAzol B preparation kit (Tel-Test, Friendswood, TX). This solution is a modification of the method of Chomczynski and Sacchi. DNA was isolated from the cell lines outlined by the Stratgene extraction kit (Stratgene Lajolla ,CA). The solutions used were based on the genomic DNA isolation method from tissue culture cells reported by S.A. Miller, et.al,. Both RNA and DNA samples were standardized to a final concentration of 1.0 µg/ul.

Slot Blot Analysis

Uniform aliquots of total RNA (0.25µg, 0.5µg, 1.0 µg) were denatured using 20X SSC, and 37% formaldehyde and heated at 60°C for 15 minutes. The samples were spotted onto nitrocellulose membrane strips using Scotlab Slot Blotter (Scotlab, Ltd. Strathclyde, Scotland) apparatus under vacuum using a pressure of 100 mm Hg. The strips were labeled and baked for two hours at 70°C . The blots were prehybridized for 10 hours at 37°C in hybridization solution (50% formamide, 6X SSC, 5X Denharts, 0.1% SDS, and 100 ug/ul denatured fragmented salmon sperm DNA). The membranes were then singly hybridized with the ^{32}P-labeled cDNA inserts corresponding to the CTPs (containing 3.0×10^7 cpm/ml) overnight in a 37°C water bath. The next day the membranes were washed once with 2X SSC/ 0.5% SDS for 5 min. at RT, 2X SSC/ 0.1% SDS for 15 min. at RT, 0.1% SSC/ 0.5% SDS for 5 min. at 37°C, 0.1X SSC/ 0.5% SDS for 45 min. at 68°C, and 0.1X SSC for 5 min. at RT. The membranes were autoradiographed for 1 to 3 days. The autoradiograms were then subjected to densitometric measurements using a Chemi Imager Tm 4000, (Alpha Inootest Corporation San Leandro, CA) to quantitate the transcription of the RNA corresponding to each probe.

Restriction Fragment Length Polymorphism (RFLP) Analysis of Genomic DNA

Genomic DNA (10µg) was digested with restriction endonucleases under conditions recommended by the manufacturers (Life Technologies, Inc. Gaithersburg, MD: Promega Corp., Madison, WI) for 2 to 4 hours. The restriction enzymes used in this study include BamH I, Hind III, Msp I, Eco RI, Xba I, Pst I and Kpn I. The digested DNA was electrophoresed on a 0.8% agarose gel for 2 to 4 hours. The gels were stained with 0.5µg/ml of ethidium bromide for 20 min. and photographed.

The agarose gels were then depurinated with 0.25mM HCl. Following depurination, the gels were then rinsed with dH_2O for 5 min. The agarose gels were then washed twice, for 30 minutes, with Solution 1 (0.5M NaOH, and 1.5M NaCl) to denature the DNA and neutralized in two 30 min. washes with Solution 2 (9.5M Tris, pH 7.5, and 3.0M NaCl). Capillary transfer for 12 hours transferred the neutralized DNA from the agarose gels to nylon membranes. After the 12-hour transfer, the nylon membranes were removed and baked for 2 hours at 70°C. The nylon membranes were then hybridized as stated above.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The RT-PCR reactions were performed in a GTC-2 thermocycler (GL Applied Research), using the Access RT-PCR system (Promega, Madison WI). The reaction mix was prepared by combining 27.5 µl of nuclease-free water, 10µl of AMV/Tfl 5X reaction buffer, 2µl dNTP mix, 1.5µl of upstream primer, 1.5µl of downstream primer, 3.0µl 25 mM $MgSo_4$, 1.0µl of AMV reverse transcriptase, 1.0µl of Tfl DNA polymerase and 3.0µl of total RNA, in a 0.50ml thin walled tube on ice. The reaction mix was then vortexed for 10 seconds to mix. The temperature cycling consisted of denaturing at 48°C for 1 minute for reverse transcription of the RNA into cDNA, 94°C for 4 minutes to deactivate reverse transcriptase, and 30 cycling sequences of denaturing at 94°C for 45 s, annealing at 45°C for 30 s, and extension at 72°C for 1 minute with a final extension at 72°C for 10 minutes. Twenty microliters of each RT-PCR reaction was electrophoresed on 1.2% agarose gels. Gels were stained with ethidium bromide and photographed using a Chemi Imager Tm 4000, (Alpha Inootest Corporation San, Leandro, CA).

TABLE 1

Established Cell Lines and Primary Cultures

Cell Lines	Characteristics	Media Supplements
1. A1N4	Mammary epithelial, immortally transformed with human oncogene	DMEM/F12, 5% FBS, insulin, EGF, transferrin, hydrocortisone, cholera toxin
2. CRL1828	Breast, normal tissue	RPMI, FBS, insulin, gentamicin
3. HS578T	Breast, ductal carcinoma	DMEM with 4.5g/L glucose, 2% FBS
4. MCF7	Breast, adenocarcinoma, pleural effusion	RPMI, FBS, 1-10 units/ml bovine insulin
5. MCF10A	Mammary gland, fibrocystic disease	DMEM/F12 + 5% FBS, 5% horse serum, EGF, Hydrocortisone, cholera toxin, insulin, penicillin/streptomycin
1. AH001	Primary colon tumor	DMEM, FBS
2. CRL1459	Colon, normal mucosa	DMEM, FBS, non-essential amino acids
3. HT29	Colon, adenocarcinoma, moderately well- differentiated grade II	DMEM, FBS, insulin, pyruvate
4. HTB37	Colon, adenocarcinoma	McCOY, FBS
5. HTB39	Colon, adenocarcinoma, ascites	McCOY, FBS, sodium pyruvate

Established cell lines and primary culture grown and analyzed for differential expression of connective tissue proteins. All media supplements were acquired from Gibco-Life Technologies.

D-MEM= Dulbecco's modified Eagle's medium
EMEM= Eagle Minimum Essential medium with Earle's salts
D-MEM/F12= Dulbecco's modified Eagle's medium : Nutrient mixture F12
FBS= Fetal Bovine Serum

Breast

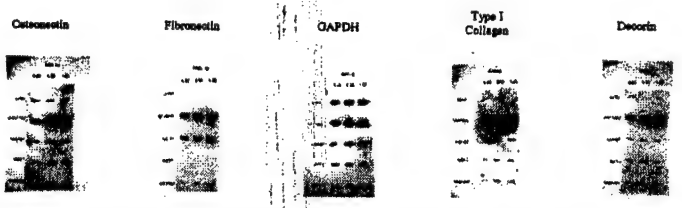

Figure 1-5. Slot blot analyses of various breast cells. (1) Osteonectin, (2) Fibronectin, (3) GAPDH, (4) Type I Collagen, (5) Decorin. Standardized amounts (0.25 µg, 0.50 µg, 1.00 µg) of total skin fibroblast RNA were denatured in 20 X SSC and 37% formaldehyde and loaded onto nitrocellulose fiber membranes mounted within the sample well platform of the Scotlab slot Blotter. The membranes were baked and hybridized with ^{32}P-radiolabeled cDNA encoding the CTPs.

Colon

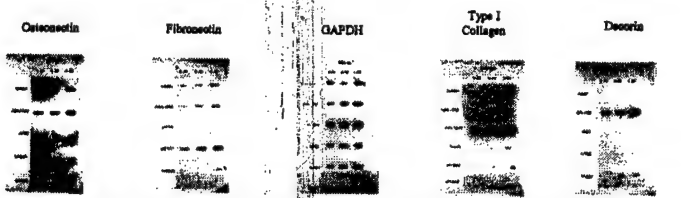

Figure 6-10. Slot blot analyses of various colon cells, (1)Osteonectin, (2) Fibronectin, (3) GAPDH, (4) Type I Collagen, (5) Decorin. Standardized amounts (0.25 µg, 0.50 µg, 1.00 µg) of total skin fibroblast RNA were denatured in 20 X SSC and 37% formaldehyde and loaded onto nitrocellulose fiber membranes mounted within the sample well platform of the Scotlab slot Blotter. The membranes were baked and hybridized with ^{32}P-radiolabeled cDNA encoding the CTPs.

Figure 11, 12. RFLP analyses of breast and colon fibroblast DNA. Two autograms of genomic DNA digested with BamH I, Msp I, Xba I, or Pst I, were electrophoresed in 0.8% agarose gel stained with ethidium bromide. Separated DNA fragments were blotted onto nylon membranes, hybridized with ^{32}P-labeled cDNA encoding osteonectin, 11 and fibronectin 12. lane 1, (A1N4); lane 2, (1828); lane 3 (HS578T); lane 4, (MCF7); lane 5, (MCF10A).

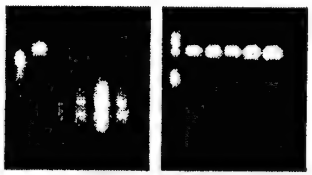

Figure 13, 14. RT-PCR analysis of breast RNA. 0.25 µg of total RNA was reversed transcribed and amplified for the desmin gene PCR 25 (13) and GAPDH (14). The products were electrophoresed in a 1.2% agarose gel stained with ethidium bromide. Lane 1, marker; 2, 1828 (3, A1N4); 4, HS578T; 5, MCF10A; 6, MCF7.

Table 2

Cell Line	Characteristics	GAPDH	TIC	DN	ON
A1N4	Mammary epithelial	83	3	24	1
CRL1828	Breast, Normal	100	100	100	100
HS578T	Breast, Ductal carcinoma	83	4	29	73
MCF7	Breast, adenocarcinoma	34	4	-0-	14
MCF10A	Mammary gland, Fibrocystic disease	59	6	18	10
AH001	Primary colon cancer	79	0	98	100
CRL 1459	Colon, Normal	100	100	100	100
HT29	Colon adenocarcinoma	102	2	-0-	30
HTB37	Colon, adenocarcinoma	90		24	23
HTB39	Colon, ascites, adenocarcinoma	91	5	32	23

Summary of densitometric analysis of slot blots

Discussion

The complexity of the process of metastasis is of great importance to cancer research and therapy. The primary determinant of metastasis is the ability of cancer cells to breach the integrity of the basement membrane. In this study, breast and colon cell lines and primary cultures were analyzed and compared for differential expression of decorin, type I collagen, osteonectin and fibronectin between cell lines and within specific cancer types.

As stated in the summary, differential transcription of all of the proteins under study was seen. The primary clinical sample, AH001 also demonstrated slightly reduced expression of these protein transcripts except decorin, which was greatly reduced in all cell types, but totally absent in this primary stage cancer. This result implies that decorin may represent the first CTP that is targeted for reduction during cancer metastasis.

Summary

1. GAPDH was expressed in all cell lines under study, indicating that cells were metabolically active.
2. Slot blot and densitometric studies demonstrated decreased expression of alpha 1 type I collagen (COLIA1). The greatest reduction in the transcription of this protein was seen in the b reast cancer cell lines.
3. There was a drastic reduction of decorin (DN) expression in both breast and colon cancer cells. The primary colon culture, AH001, demonstrated no measurable expression of this protein.
4. Osteonectin (ON) expression was reduced 4-fold in breast cancer cells and 3-fold in colon cancer cells.
5. Fibronectin (FN) was only slightly reduced in HS5167T (ductal carcinoma) but drastically reduced in all other breast and colon cells under study.
6. Adenocarcinoma of both breast (MCF7) and colon (HT29) displayed no detectable transcription of the FN gene.
7. The primary cancer tissue, AH001, demonstrated near normal transcription levels of the CTP proteins, except decorin, whose transcript was totally absent in these cells.
8. RFLP analysis of the breast samples revealed polymorphic shifts in the migration of the osteonectin gene DNA from MCF7 and MCF10A when digested with MspI.
9. RFLP of colon samples revealed polymorphic differences in DNA from HT29, HTB37 and HTB39 for the FN gene.

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STUDY OF THE EFFECT OF OMEGA-3 AND OMEGA-6 FATTY ACID ON CELL GROWTH AND OXIDATIVE STRESS IN BREAST CANCER CELLS

Rasha Hammamieh, Kate carr, Carrie Dulany, Rina Das and Marti Jett

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ABSTRACT: Epidemiological and preclinical studies have indicated a relationship between dietary practices and development of cancer. Lipid content and subsequently the derived fatty acid composition of the diet are believed to play a major role in the development of tumorigenesis. Omega-3 fatty acids, including long chain polyunsaturated fatty acids (n-3 PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), can effectively reduce the risk of cancer whereas omega-6 fatty acids such as arachidonic acid (AA) and linoleic acid (LA) reportedly promote risk. To investigate the effects of fatty acids on tumorigenesis, we performed experiments to examine the effects of the omega-3 fatty acids EPA and DHA and of the omega-6 fatty acids AA and LA on cell growth in MDA-MB-231 breast cancer cells. We also studied the effect of omega-3 and omega-6 fatty acids on oxidative stress in MDA-MB-231. Furthermore, the effect of omega-6 fatty acids on gene expression of fatty acid binding proteins, PPAR-g and other genes involved in cells regulations and oxidative stress were studied. Our results showed that omega-3 fatty acids inhibited cell proliferation in breast cancer cells while omega-6 promoted cell proliferation. When TBARS assay was used to study oxidative stress, Omega-3 fatty acids showed an increase in oxidative stress compared to omega-6 fatty acids.

ANTI-SENSE OLIGODEOXYNUCLEOTIDE COMPLEMENTARY TO LIVER FATTY ACID BINDING PROTEIN ALTERS CELLULAR FUNCTIONS IN BREAST CANCER CELLS

Rasha Hammamieh, Ryan Thomas, Rina Das and Marti Jett

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ABSTRACT: Studies show a relationship between dietary fat intake and increased incidence and growth of hormonally regulated cancers, such as breast and prostate. Dietary fats, especially arachidonic acid, are readily metabolized into potent bioactive lipids that stimulate proliferation in these cancer cells. The action of these lipids may be mediated by a family of small intracellular proteins, fatty acid binding proteins (FABP's), named according the tissue from which they were first identified. Our previous results showed that Liver(L)- and Intestine-FABPs were elevated in MCF-7 and T47D cancer relative to other breast cancer cells and normal breast cells. However, Epidermal (E)- and Adipose-FABPs were down-regulated in breast cancer cells compared to normal cells. Therefore, we developed antisense oligodeoxynucleotides able to direct RNAse H activity on full-length L-FABP mRNA. Addition of anti-L FABP oligodeoxynucleotide caused a significant decrease in the growth rate of breast MCF-7 tumor cell lines. Furthermore, anti-L FABP, at 10 micro molar, induced apoptosis in breast MDA-MB-435 and MCF-7 cancer cells. To further understand the mechanism of action of anti-L FABP in cancer cells, we used quantitative RT-PCR and human cDNA array blots to explore differentially expressed genes in breast cancer cells treated with anti-L FABP antisense. This analysis revealed alterations in some of the genes that are significantly involved in the regulation of cell growth and apoptosis. These data support the contention that certain FABPs correlate with tumorigenicity. This study will provide the basis for a better understanding the direct role of fatty acids/bioactive lipids and FABPs in cancer development and progression, and indicates the therapeutic potential for FABPs to serve as targets for treatment of these hormonally-regulated cancers.

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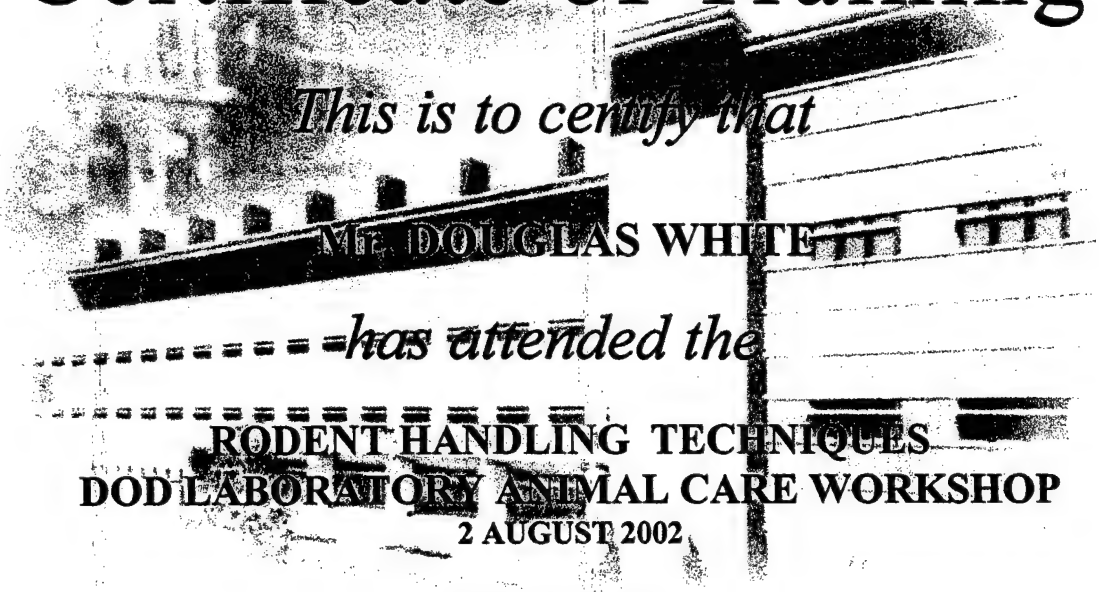

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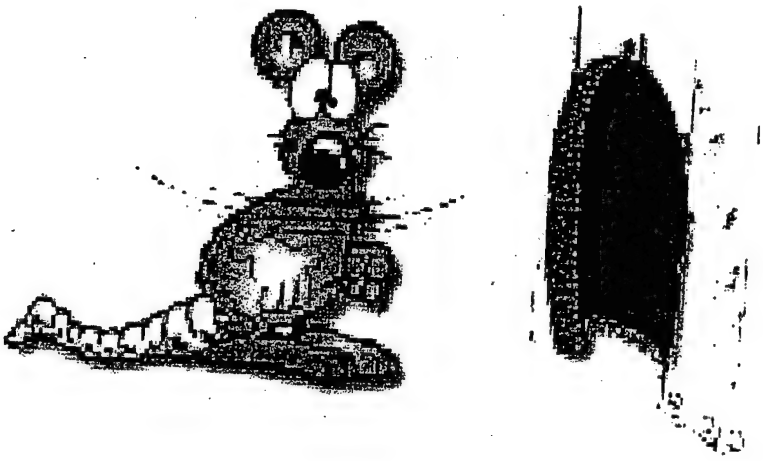

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***The Guide for the Care and Use of Laboratory Animals* (1996)**
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(202)334-3313
The Guide can be found at http://www.nap.edu/readingroom/books/labrats

TABLE OF CONTENTS

HANDLING TECHNIQUES

MOUSE: Mice are small rodents that are hard to grasp. Care should be taken to avoid getting bit or causing harm to the animal. One method of moving mice from one cage to another is by grasping the skin behind the neck with a pair of forceps. When using this method be careful not to grasp too hard. Restraining the mouse can be done by grasping the base of the tail with one hand and with the other grasp the loose skin behind its neck. Take extra precaution to avoid getting bitten. When you have a firm grasp you may secure the tail in the same hand you have the scruff in to accomplish a one handed restraint.

RAT: Rats can inflict a painful bite. **DO NOT** grasp the rat by the scruff, as it will react violently to this type of restraint. First grab the rat by the base of the tail and lift out of the cage and place on a soft surface. Hard smooth surfaces can make the rat tense. Second, become friends with your rat by gently petting it, as this will calm it. Place your index and middle fingers along side the rat's head and your thumb and ring fingers under its forelegs. Use your index and middle fingers to secure its head and the remaining fingers to support the chest. Hold the tail and support the lower body with you other hand. Be careful not to squeeze hard as this may interfere with the rat's ability to breathe.

GUINEA PIG: Guinea pigs are curious, easy to handle animals. They are not aggressive by nature. Do not grasp the guinea pig by the loose skin. Body hair is easily pulled out and the guinea pig will often object when handled in this manner. Calmly grasp it with one hand under the chest and use your other hand to support its hindquarters.

***Care should be taken not to excite any of these animals. Slow deliberate movements will make the job of restraining much easier. Noise should also be kept to a minimum.

SEX DETERMINATION

MOUSE: Restrain the mouse and lift the base of the tail. Sex is most easily determined by ano-genital distance. Males normally have a greater distance between the anus and urogenital openings. Male mice also have a larger genital papilla.

RAT: Procedure same as in the mouse.

GUINEA PIG: Both male and female guinea pigs display similar ano-genital distances. The female has a separate urethal orifice, a vaginal membrane, a perineal sac, and an anus; the male has a penis, a larger perineal sac, and an anus. The penis lies just under the skin and can be inverted with gentle pressure. The testes and penis are palpable in adults

ORAL GAVAGE

MOUSE: Restrain the mouse and measure the gavage tube from the tip of the nose to the last rib. This is the length you must insert the tube. With the use of the tube push the mouse's head slightly upward and back to straighten the esophagus. Position tube to the right or left of the mouth and slowly pass the tube watching for the swallowing reflex. The tube should pass freely into the esophagus. DO NOT FORCE. When desired length of insertion is achieved, inject solution. Observe mouse after the procedure for signs of distress, such as gasping or frothing as the mouth.

RAT: Procedure same as in the mouse.

GUINEA PIG: The guinea pig has a small palatal ostium that is easily damaged. For this reason this procedure is not recommended in the guinea pig.

*** When using a plastic tube, care should be taken to ensure animal does not bite down and sever the tube. An artificial device to hold the mouth open can be used.

INJECTION TECHNIQUES
GENERAL OVERVIEW

MOUSE: Injection sites should be cleaned with a suitable disinfectant, typically isopropyl alcohol. Sterile syringes and needles must be used for all injections. The one time use of disposable supplies insures aseptic techniques and sharp needles. Always select the smallest gauge needle possible to limit tissue trauma and injection discomfort. A 25–30 gauge needle is recommended for use in a mouse. Before injecting, check for correct placement by pulling back on the plunger of the syringe to create a vacuum. This is known as aspiration.

RAT: Same overview as the mouse except it is recommended that 21-30 gauge needles be used.

GUINEA PIG: Same overview as the mouse except it is recommended that 22-30 gauge needles be used.

SUBCUTANEOUS (SQ) INJECTION

MOUSE: The mouse should be restrained in the normal manner. With your fingers, lift the skin to make a "tent." Disinfect the injection site and insert needle into the subcutaneous tissue. Aspirate prior to making the injection. Proper placement should yield no aspirate. Inject.

-Most common injection site is the loose skin around the neck and shoulder area.

RAT: Restrain the rat by grasping the base of the tail with one hand and with the other, flatten the rat against the table. With your fingers, lift the skin to make a "tent." Disinfect injection site and insert needle into the subcutaneous tissue. Aspirate, you should not aspirate anything. Inject.

- Most common injection site is the loose skin around neck and shoulder area.

GUINEA PIG: The guinea pig should be restrained and the injection site disinfected. With your fingers, lift the skin to make a "tent." Insert needle into the subcutaneous tissue and aspirate, you should not aspirate anything. Inject.

-Most common injection site is the loose skin around neck and shoulder area

*** The recommended needle size for SQ injections are 23-25 gauge.

INTRAMUSCULAR (IM) INJECTION

MOUSE: IM injections are not recommended due to the mouse's lack of muscle mass. Injection may cause discomfort and local tissue irritation.

RAT: Restrain rat by either holding rat against your body and isolating the rear leg or restrain the rat in the traditional manner and grasp the hind leg and hold securely. Disinfect injection site and insert needle into the caudal thigh muscle. You first must isolate the caudal thigh muscle to prevent injection into the ischiatic nerve. Injection into the nerve may cause discomfort and lameness. Aspirate and inject. If blood is aspirated, you must reposition the needle.

GUINEA PIG: Restrain the guinea pig and isolate the caudal thigh muscles. Disinfect injection site and insert the needle. Aspirate. Prior to making the injection be sure to inject into the caudal thigh. Placement of the needle too far laterally can result in damage to the ishiatic nerve. Another injection site is the lumbar muscles. To administer an IM injection here, outline the lumbar muscles with your thumb and second finger, using your index finger locate the vertebral column for orientation. Insert needle lateral to the midline, avoiding the spine.

*** The recommended needle size for an IM injection is 25 gauge.

INTRADERMAL (ID) INJECTIONS

MOUSE: ID injections are not commonly performed in the mouse due to limited clinical application.

RAT: ID injections are not commonly performed in the rat due to limited clinical application.

GUINEA PIG: Restrain the guinea pig by using either physical or chemical restraint. Shave or Nair the injection site and disinfect. Isolate the injection site by pinching or stretching skin. Insert the needle bevel up just under the surface of the skin and inject. A distinct bleb should form.

*** The recommended needle size for an ID injection is 25 gauge.

INTRAPERITONEAL (IP) INJECTION

MOUSE: Restrain the mouse and tilt so that the head is facing downward and its abdomen is exposed. Disinfect injection site and insert the needle cranially into the abdomen at a 30-45 degree angle caudal to the umbilicus and lateral to the midline.

Aspirate

-greenish-brown aspirate indicates needle penetration into the intestines

-yellow aspirate indicates needle penetration into the bladder

If any fluid is aspirated, your solution is contaminated and must be discarded and the procedure repeated with a new syringe and needle. If nothing is aspirated, inject. The recommended needle size for IP injections in the mouse is 25-27 gauge.

RAT: Procedure same as in the mouse
The recommended needle size for IP injections in the rat is 25 gauge.

GUINEA PIG: Procedure same as in the mouse. The recommended needle size for IP injections in the guinea pig is 23-25 gauge.

INTRAVENOUS (IV) INJECTION

MOUSE (Tail Vein): Restrain the mouse with physical or chemical restraint. Rotate the tail slightly to visualize vein. Disinfect injection site and insert needle (27-30 gauge) into the vein at a slight angle. You will not be able to aspirate, instead inject slowly and watch for clearing of the lumen. Incorrect positioning will result in a slight bulge in the tail. If this occurs, remove needle and repeat process proximal to previous site. Upon completion remove needle and apply pressure to injection site.

RAT (Tail or Saphenous Vein): Tail injection procedure same as in the mouse. For the saphenous vein, restrain the rat with the use of anesthesia. Extend the hind leg and shave hair to expose lateral saphenous vein. Disinfect the injection site and apply tourniquet-like pressure to the upper portion of the leg. Insert needle into the vein and aspirate. Release tourniquet pressure and inject. Upon completion remove needle and ensure proper hemostasis. The recommended needle size for IV injections in the rat is 22- 25 gauge.

GUINEA PIG (Saphenous Vein): Same procedure as for the rat, but much more difficult to visualize in the guinea pig. The recommended needle size for IP injections in the guinea pig is 25 gauge.

***Be sure there are no air pockets or bubbles in the solution to be injected, as this can kill small animals.

BLOOD WITHDRAWAL TECHNIQUES
GENERAL OVERVIEW

MOUSE: Withdrawal sites should be cleaned with a suitable disinfectant. Sterile syringes and needles must be used for all withdrawals. The one time use of disposable supplies insures aseptic techniques and sharp needles. Always select the smallest gauge needle possible to limit tissue trauma and discomfort. A 25–30 gauge needle is recommended for use in a mouse. Check for correct placement by pulling back on the plunger of the syringe to create a vacuum. This is known as aspiration.

RAT: Same overview as the mouse except it is recommended that 21-30 gauge needles be used.

GUINEA PIG: Same overview as the mouse except it is recommended that 22-30 gauge needles be used.

TAIL ARTERY AND VEINS

MOUSE: The tail arteries and veins may be used to collect small amounts of blood. First warm the animal in an incubator or under an incandescent light. The animal must be watched closely during this procedure to ensure they don't overheat or receive thermal burns to their extremities, particularly their ears. There are two blood collection techniques that can be used.

-Restrain mouse, disinfect withdrawal site and insert a 25 gauge needle into the vein and use a capillary tube to collect the blood from the hub.

-Restrain mouse in a rodent restrainer, disinfect withdrawal site and locate vein. Using a razor blade, knick tail vein and collect in a blood collection tube or capillary tube. This method should only be used every 1-2 weeks for a limited number of collections.

Upon completion of either blood withdrawal technique, ensure proper hemostasis

RAT: The tail arteries and veins may be used to collect small amounts of blood. First you must warm the animal in an incubator or under an incandescent light. Restrain rat in a rodent restrainer, disinfect withdrawal site and insert a 25 gauge needle into the vein. Use a capillary tube to collect the blood from the hub. Upon completion apply pressure until bleeding stops.

GUINEA PIG: This method of blood collection is not recommended in the guinea pig, as the guinea pig has no tail.

PERIORBITAL VENOUS SINUS
ORBITAL VENOUS PLEXUS

MOUSE (periorbital venous sinus): This method must be done on an anesthetized mouse and is only recommended to be done at weekly intervals using alternate eyes. Microhematocrit tubes or Pasteur pipettes may be used to collect blood. Push the upper and lower eyelids apart to protrude the globe. Insert the tube into the medial canthus of the eye. Apply slight downward pressure while rotating the tube to pass it through the conjunctiva and into the periorbital sinus. When the tube "pops" through, back tube out slightly to allow blood to flow. When collection is complete, close both eyelids and apply pressure with a piece of gauze until bleeding stops.

RAT (orbital venous plexus): This method must be done on an anesthetized rat and is only recommended to be done at weekly intervals using alternate eyes. Microhematocrit tubes or Pasteur pipettes may be used to collect blood. Push the upper and lower eyelids apart to protrude the globe. Insert the tube into the dorsal portion of the bony orbit. Apply slight downward pressure while rotating the tube to pass it through the conjunctiva and into the venous plexus. When the tube "pops" through, back tube out slightly to allow blood to flow. When collection is complete, close both eyelids and apply pressure with a piece of gauze until bleeding stops.

GUINEA PIG: This method of blood collection is not recommended in the guinea pig.

EAR PRICK

MOUSE: This procedure is not used in the mouse.

RAT: This procedure is not used in the rat.

GUINEA PIG: Restrain the guinea pig. The use of a towel is helpful in restraining. Disinfect the ear. With a 22 gauge needle insert it perpendicular into the vein and remove. Collect blood in a microhematocrit tube or Pasteur pipettes. When collection is done ensure proper hemostasis.

CRANIAL VENA CAVA

MOUSE: This procedure is not used in the mouse.

RAT: This method must be done on an anesthetized rat. It is recommended that a 3cc or 6cc syringe with a 22-23 gauge needle be used. Place the rat in dorsal recumbency and locate the cranial portion of the sternum. Insert the needle at a 30-45 degree angle, under the first rib, lateral to the sternum on the animal's right side. Direct the needle toward the midline and insert it no more than 1/4 inch. Maintain a slight amount of negative pressure and slowly withdraw until blood begins to flow. After the procedure is complete, remove needle and apply pressure to the injection site. If no blood flows, remove needle and repeat procedure. Probing for the vessel is not recommended as this could cause major damage and premature death. This procedure is useful in the rat for collecting 1ml or less of blood.

GUINEA PIG: Procedure same as in the rat. This procedure is useful in the guinea pig for collecting 1 1/2ml or less of blood.

CARDIAC PUNCTURE

MOUSE: This method must be done on an anesthetized mouse and is only recommended to be done as a terminal procedure. The use of a 1cc syringe with a 25 gauge needle is recommended. Find the xiphoid process as a reference point. Insert the needle at a 35-40 degree angle just under and to the left of the xiphoid process. As the needle is inserted into the chest, gently aspirate until blood begins to flow. Overzealous withdrawal may collapse the heart. If you do not get blood flow on the first try, withdraw the needle and repeat entire process. Probing for the heart is not recommended, this could cause damage to major vessels and premature death.

RAT: Procedure same as in the mouse, except it is recommended that a 6cc or 12cc syringe with a 20-22 gauge needle be used.

GUINEA PIG: Procedure same as in the mouse, except it is recommended that a 12cc or 20cc syringe with a 20-22 gauge needle be used.

Upon completion of this procedure the animal should be euthanized and disposed of properly.

Humane Animal Care and Use

All animals owned by the U.S. Army for research or training will receive proper care, and will be used humanely in accordance with approved protocols, federal laws, and Department of Defense regulations and guidelines. Any person who witnesses or suspects abuse of animals is encouraged to report their concern to:

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This teleconference will explore four of the nine core instructional areas defined as Responsible Conduct of Research:

- human subjects research
- conflict of interest
- research misconduct
- mentorship

Panel of Experts: (Partial list)

Greg Koski, PhD, MD, Director, Office of Human Research Protection, NIH

David Korn, MD, Sr. VP, Div. of Biomedical & Health Sciences Research, American Association of Medical Colleges

Joan Porter, DPA, MPH, Asso Dir, Office of Research Compliance & Assurance, Veterans Administration

Chris Pascal, Director, Office of Research Integrity, HHS

Mark S. Frankel, Program Director, AAAS

Geoff Grant, Asso VP, Stanford University Research Administration

Co-sponsored by the Howard University Cancer Center and the DOD-HUCC/WRAIR Training Grant # DAMD 17-01-1-0268. For more information call Colleen Sundstrom at 202-806-7037.

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Agnes Day, PhD
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Conducting Research Responsibly Teleconference

Thursday, September 13, 2001

Attendance

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Sponsored by Howard University Cancer Center and DOD HU/WRAIR Training Grant # DAMD 17-01-1-0268

John T. Stubbs III, Ph.D.
Breast Cancer Project

Breast Cancer in African American Women: Molecular Analysis of Differences in Incidence and Outcomes.

Abstract

Bone sialoprotein (BSP) is a small integrin binding phosphosialoprotein that has the ability to bind to cells. BSP also has the ability to bind to and de novo nucleate hydroxyapatite, the mineral found in bone. BSP is normally synthesized by osteoblasts, osteoclasts, and chondrocytes. Ectopic BSP expression in primary breast tumors has been correlated with 1) later development of breast cancer bone metastasis and 2) poor patient survival. Furthermore, in vitro studies suggest that exogenous bone sialoprotein can induce the proliferation, migration, and cell adhesion of breast cancer cells.
Collectively, this data suggest that BSP plays some role in breast cancer. However, it is unknown if BSP is essential for breast tumor survival and or osteotropism. We propose to utilize an inducible expression system to investigate the biological effects that BSP has upon breast cancer cells in vitro and in vivo. Conditional BSP expression in breast cancer cells allows direct correlation between BSP expression and biological parameters associated with metastasis since BSP induced effects should appear when BSP is induced and disappear when BSP is not stimulated. Additionally, an inducible expression system allows one to investigate the biological down stream effects of BSP gene expression at different stages of tumor development. We have partnered with Drs. Marti Jett and Rina Das of the Walter Reed Army institute of Research in the Howard University / Walter Reed breast cancer training program entitled Breast Cancer in African American Women: Molecular Analysis of Differences in Incidence and Outcomes. One of the many exciting components of this program is the non-invasive in vivo imaging technologies that may be utilized to monitor the growth and distribution of tumor cells in live rodents. Presently, we are constructing BSP (and BSP related extracelular matrix proteins) inducible vectors that will be used to transform and generate BSP inducible breast cancer cell lines. Ultimately, these cell lines will be used in animal models to study the effects of induced BSP expression on breast tumor growth, distribution, and osteotropism.

SELECTIVE ESTROGEN RECEPTOR MODULATORS AND DEPRESSION

A proposal submitted to Howard University Cancer Center

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SPECIFIC AIMS

Extensive interactions between estrogen and central neurotransmitter systems controlling the mood are documented. The effects of estrogen are mediated primarily through estrogenic receptors that are expressed in distinct brain regions involved in regulation of affective behavior. Adequate stimulation of the estrogenic receptors appear to be essential in mood regulation. Inadequate stimulation of ERs due to reduction of estrogen levels (e.g. during menopause or post-partum) may trigger depressive feelings in some women. Similarly, blockade of estrogen receptors by antiestrogenic drugs may also result in depression. Tamoxifen is an oral anti-estrogen, first generation selective estrogen receptor modulator (SERM) that is widely used for the treatment of breast cancer. While tamoxifen is generally well tolerated, some serious side effects including anxiety and depression may be associated with its use. Raloxifene is a new, second generation SERM with clinical profiles similar to tamoxifen. Although raloxifene has not yet been recommended for breast cancer treatment, it has promising therapeutic potential in reducing the risk of breast cancer in postmenopausal women treated for osteoporosis. The effects of raloxifene on mood or affective behavior is not known. In this proposal, using animal models, we plan to further elucidate the relationship between estrogen/antiestrogens and mood regulation.. Specifically, we will determine: 1. whether estrogen may exert antidepressant effects in an animal model of depression; 2. whether depressive characteristics in an animal model of depression is associated with low levels of estrogen; 3. whether antiestrogenic compounds such as tamoxifen or raloxifene would induce depressive characteristics in an animal model. In

addition, to gain a better understanding of the interaction of estrogenic compounds with central neurotransmitter systems implicated in mood regulation, the effects of estrogen and its antagonists on several biogenic amines will be evaluated. This will be achieved by measuring the release of dopamine, serotonin and norepinephrine in distinct brain regions following chronic administration of estrogen or its antagonists. The results of these studies would not only enhance our understanding of the relationship between estrogen/estrogenic receptors and mood regulation but would also be of relevance to treatment of breast cancer with SERMs.

BACKGROUND

SERMs

Recent advances in the molecular pharmacology of estrogen and estrogen receptors have resulted in the development of agents that are selective estrogen receptor modulators (SERMs).These compounds posses very selective estrogenic activities (both inhibitory and stimulatory) and their effects are tissue-specific. Thus, in the breast SERMs act as estrogenic antagonists to inhibit estrogen-induced proliferation of cancer cells, whereas in the endometrium, bone, brain and liver SERMs may act as estrogenic agonists (see below).

TAMOXIFEN AND BREAST CANCER

Tamoxifen (nolvadex®, Zeneca, Pharmaceuticals, Wilmington, DE) is an oral anti-estrogen, first generation selective estrogen receptors modulator that is widely used for the treatment of breast cancer. Breast cancer is the most common and frequently diagnosed cancer and the major cause of cancer related deaths in women (Greenlee et al. 2000). In the United States, approximately 183,000 women are diagnosed with invasive form of breast cancer annually and nearly 41,000 die of the disease every year (Greenlee et al. 2000; Bennett et al. 2000). Tamoxifen was originally synthesized in 1966 by Harper and Walpole, in England as an

antifertility drug. Evaluation of tamoxifen for the treatment of breast cancer was started in 1970. It was approved in the U.S. in 1997 and is the most frequently prescribed endocrine therapy for women with breast cancer (Robinson et al. 1996; Assikis and Jordan, 1997). The use of tamoxifen has expanded to include management of breast cancer in premenopausal women, systemic adjuvant therapy for early stage breast cancer in pre- and postmenopausal women, and treatment of advanced breast cancer in both men and women, especially for cancer cells that are positive for estrogen receptors (Robert, 1997). Thus, tamoxifen may be efficacious in the treatment of all stages of breast cancer (Jordan, 1993; Jordan and Murphy,1990). Tamoxifen may also prevent osteoporosis (Thomson et al.1999; Wilseman and Lewis, 1996).

SIDE EFFECTS OF TAMOXIFEN

While tamoxifen is generally well tolerated, some serious side effects may be associated with its use. The most frequent side effects of tamoxifen include flashes, nausea, vomiting, fatigue, thrombophlebitis, endometrial cancer, anxiety and depression (Arnold et al 2001). Other vasomotor and gynecological symptomatic side effects may also be frequent (Love, 1992). Whereas flashes may be attributed solely to tamoxifen use, depression, anxiety and fatigue may also be attributed to breast cancer itself.

TAMOXIFEN, ANXIETY AND DEPRESSION

Many reports indicate that women with breast cancer have an increased incidence of anxiety and depression (Brever and Anderson, 2000; McDaniel et al. 1995; Feting 1997; Moyer and Salovery, 1996). In addition, several reports indicate possible association of anxiety and/or depression with tamoxifen treatment (Cathart et al., 1993; and Shariff et al. 1995). Thus, the incidence of depression in breast cancer patients may be exacerbated by tamoxifen therapy (Brever and Anderson, 2000).

ESTROGEN AND ESTROGENIC RECEPTORS

Estrogens are steroid hormones that play an important role in the growth and development of the mammary gland, uterus, vagina, and, the ovary. Estrogen is responsible for the changes that take place at puberty and development of the secondary sexual characteristics in females. The action of estrogen is mediated through binding to an intracellular estrogen receptor (ER) (Beato, 1989; Evans, 1988), which undergoes extensive conformational changes upon ligand binding (Renaud et al 1995; Wagner et al 1995). The ER is a DNA binding protein that is a member of the super family of nuclear receptors that includes the steroid hormones, Vitamin D, thyroid hormone, and retinoic acid receptors. Although these receptors have considerable variation in molecular size, they share a common functional and structural organization.

Two major estrogen receptor subtypes have been identified. One is the classic ER, or estrogen receptor alpha, ERα (Greene et al, 1986; Greene et al, 2000) and the second is estrogen receptor beta, ERβ that was recently identified and cloned from both human and rat tissue (Kuiper et al, 1996; Mosselman et al, 1996). These two receptor subtypes appear to have distinct anatomical distribution as well as physiological functions. Kuiper et al (1996) reported that there is more expression of ERα in the uterus, testis, pituitary and epididymis, while ERβ appeares to be dominant in the prostate and bladder tissues. ERα have been detected in different brain regions: frontal cortex, hippocampus, hypothalamic nuclei, nucleus accumbens (Fink et al.,1996; Shughrue et al.1997; Holschneider et al. 1998; Chen et al. 2002). Melinda et al (2002) reported that ERα is highly concentrated in the anteroventral periventricular, medial preoptic, arcuate, and ventromedial nuclei and the amygdala, regions of the brain that are responsible for reproductive functions; whereas, ERβ is concentrated in the cerebral cortex, hippocampus,

periventricular preoptic, preoptic, the striatum, paraventricular and supraoptic nuclei, and amygdala. There is an equal distribution of both subtypes in the breast and the ovary. In addition to estrogen, other agents may also activate the ER. These include: epidermal growth factors (EGF), insulin-like growth factors (ILGF) and neurotransmitters such as dopamine (Aronica & Katzenellenbogen 1993; Ignar-Trowbridge et al 1993; El-Tanani et al 1997; Smith et al 1993). Activation of the ER may be mainly through phosphorylation pathways involving protein kinases (Trowbridge et al., 1993; Kato et al., 1995).

TAMOXIFEN AND ESTROGEN RECEPTORS

Although the mechanism of action of tamoxifen is not yet clearly understood, its primary effect is believed to be through binding to the estrogen receptors and acting mainly as a competitive estrogen inhibitor. Tamoxifen may act by altering ER structural conformation or by altering the RNA transcription (Thompson et al 1999; Jackson et al 1997). Thus, in breast cancer cells tamoxifen blocks the binding of estrogen to its receptors and prevents estrogen-dependent cancer cells from growth and multiplication. This results in inhibition of proliferation of breast cancer cells and causes reduction in tumor size and number (Jiayesimi et al.1995). This effect of tamoxifen renders it as one of the most effective non-steroidal anti-estrogen agents for the treatment of ERα positive breast cancer (Wijayarantne et al. 1999).

In the endometrium, bone and lipids, however, tamoxifen may act as an estrogen agonist. Estrogenic action of tamoxifen in the endometrium can result in proliferation of endometrial cells and lead to endometrial cancer (Lahti et al. 1993). The estrogenic activity of tamoxifen in bones and lipids, on the other hand, may be associated with positive effects in preserving mineral density and lipid profiles. This suggests that tamoxifen may be a suitable alternative to traditional estrogen replacement therapy in conditions such as postmenopausal-

related osteoporosis. An added advantage for this recommendation would be the avoidance of an increased risk of breast cancer which may follow long-term estrogen therapy (Jordan 1989).

Tamoxifen may also cause apoptosis of potentially malignant cells by increasing production of transforming growth factors, decreasing insulin-like growth factors and/or increasing circulating levels of serum hormone binding globulin (SHBG) Vogel (1995). Increase in levels of SHBG may decrease the availability of estrogen. The agonist or antagonist effects of SERMs are mediated through transcription activation functions (AF-1 and AF-2) in the ER. The AF-1 domain appears to be essential for the agonistic activity of SERMs (Berry et al 1990; Tzukerman et al 1994; Webb et al, 2000), while AF-2 domain may be responsible for the antagonist activity of SERMs (Chakravarti et al 1996; Yao et all 1996).

RALOXIFENE

Raloxifene (Evista ®), is a new, second generation selective estrogen receptor modulator (SERM) (Body and Sternon, 2000). It is a benzothiophene derivative with clinical profile similar to tamoxifen. Raloxifene has been approved for the treatment and prevention of osteoporosis in postmenopausal women. Raloxifene has antiestrogenic effects on breast and endometrial tissues; and estrogenic effects on bone, lipid metabolism and blood clotting. It lowers the blood levels of total and low-density lipoprotein the "bad cholesterol,"but doesn't affect the concentration of high-density lipoprotein (Agnusdei, 1999). Raloxifene, unlike tamoxifen does not cause estrogenic effects in the uterus (Goldstein et al. 1999). Although raloxifene has not yet been recommended for breast cancer treatment, it has promising therapeutic potential in reducing the risk of breast cancer in postmenopausal women treated for osteoporosis (van den Brule et al. 1999;Goldsteinet al. 2000; Yao and Jordan ,2000).

Tamoxifen and raloxifene are under investigation by the National Surgical Adjuvant Breast and Bowel Project (NSABP) to evaluate how the drug raloxifene compares with tamoxifen in reducing the incidence of breast cancer in women who are at high risk of developing the disease. Both tamoxifen and raloxifene interact with both ER subtypes, but the two SERM recognize different surfaces on ERα and ERβ and each ligand induces a distinct pharmacological effect (Erikensen, 2000).

ESTROGEN, MOOD AND CENTRAL NEUROTRANSMISSION SYSTEMS

Effects of estrogen on mood, mental state and memory have been reported (George et al. 1996; Fink et al 1996). Indeed, it has been postulated that low levels of estrogen may be a contributing factor to postmenopausal depression (Halbreich 1997). Estrogen may interact with serotonin (5HT), dopamine (DA) and norepinephrine (NE) (Biegon and McEwen 1982; Dluzen 2000; Fink et al 1996; Thompson et al 1999), central neurotransmitters that have been implicated in mood regulation (see below).

BIOGENIC AMINES AND MOOD REGULATION

Alterations in a number of neurochemicals, particularly biogenic amines (e.g. norepinephrine, dopamine and serotonin) have been postulated as etiologic factors in affective disorders (see recent reviews: Ban 2001, Bauman and Bogerts 2001, Pacher et al 2001, Skolnick et al 2001, Sampson 2001). Indeed, Current pharmacotherapy of depression is primarily based on pharmacological alterations of one or another biogenic amine (see reviews by Skolnick et al 2001, Sampson 2001).

Although the exact circuitry mediating mood regulation remains unknown, specific biogenic amine pathways have been implicated in antidepressant effects of current

pharmacotherapies. These include mesolimbic and mesocortical dopamine system, locus coeruleus-frontal noradrenergic pathway, dorsal raphe-(prefrontal cortex and amygdala), and medial raphe-hippocampus systems (Drevets 1998, Sheline et al 1998, Balfour and Ridleyl 2000, Quattrocki et al 2000, Bauman and Bogerts 2001, Linner et al 2001, Zangen et al 2001).

ESTROGEN AND SEROTONIN (5HT)

The serotonin system plays a pivotal role in the regulation of mood, affective behavior, pituitary hormone secretion and many autonomic activities. The amino acid tryptophan is the primary substrate and the enzyme tryptophan hydroxylase is the rate-limiting enzyme for the synthesis of 5-hydroxy tryptophan (serotonin). Depletion of serotonin in the mammalian central nervous system has been reported to induce profound behavioral depression (Cooper et el. 1996). Estrogen increases 5-HT2A receptors in several brain regions including: anterior frontal, cingulated and primary olfactory cortex, and nucleus accumbens (Fink et al 1996). Because estrogen can modulate mood through its interaction with serotonin (Chang and Chang 1999; Fink et al. 1996), the antiestrogenic drug, tamoxifen may also have mood altering effects (Julia et al. 2001).

ESTROGEN AND DOPAMINE (DA)

Dopamine (3,4-dihydroxyphenylethylamine) is a major monoamine neurotransmitter that represents about 50 % of total catecholamines of the central nervous system of most mammals. The dopaminergic system is believed to play important roles in emotional responses (mood, pain, pleasure), motor control, and endocrine functions (Clark et al.1987; DiChiara, 1995; Fremeau et al. 1991; Graybiel et al. 1994; Kiyatkin, 1995). Modulation of dopamine receptors, dopamine release, dopamine synapses, dopamine neuron differentiation as well as neuroprotection of dopamine system by estrogen have been reported.

ESTROGEN AND NOREPINEPHRINE

Norepinephrine (NE) is derived from DA through enzymatic process that involves dopamine-beta-hydroxylase. It is well established that NE exerts important role in diverse neuronal networks involved in regulation of variety of functions including affective behavior (Ban 2001, Bauman and Bogerts 2001, Pacher et al 2001, Skolnick et al 2001, Sampson 2001). Stimulatory effects of estrogen on NE turnover and NE-induced cAMP increase have been documented (Hiemke et al 1985; Etgen 1987). In addition, it is now known that selective sub-population of brainstem NE neurons express ERs and that estrogen can modulate NE transmission by regulating adrenergic receptor expression and function in distinct brain regions including the cortex (Herbison et al 2000).

GENERAL AIMS

The above discussions suggest extensive interactions between estrogen and central neurotransmitter systems controlling the mood. The effects of estrogen are mediated primarily through estrogenic receptors that are expressed in distinct brain regions involved in regulation of affective behavior. Adequate stimulation of the estrogenic receptors appear to be essential in mood regulation. Inadequate stimulation of ERs due to reduction of estrogen levels (e.g. during menopause or post-partum) may trigger depressive feelings in some women. Similarly, blockade of estrogen receptors by antiestrogenic drugs may also result in depression. In this proposal, using animal models, we plan to verify some of the above postulates. Specifically, we will determine: 1. whether estrogen may exert antidepressant effects in an animal model of depression; 2. whether depressive characteristics in an animal model of depression is associated with low levels of estrogen; 3. whether antiestrogenic compounds such as tamoxifen or

raloxifene would induce depressive characteristics in an animal model. In addition, to gain a better understanding of the interaction of estrogenic compounds with central neurotransmitter systems implicated in mood regulation, the effects of estrogen and its antagonists on several biogenic amines will be evaluated. This will be achieved by measuring the release of dopamine, serotonin and norepinephrine in distinct brain regions following administration of estrogen or its antagonists.

RESEARCH DESIGN AND METHODS

Specific Aims 1.

Objective:

Determine whether acute or chronic estrogen may exert antidepressant effects in an animal model of depression.

Protocol

The Wistar Kyoto (WKY) rat model of depression will be used to evaluate the possible antidepressant effects of estrogen. WKY rat has been proposed as a suitable model for studying the biological substrates of depression (Paré 1989, 1994, Marti and Amario 1996). WKY rats show considerable reduction in locomotor activity in the open-field and exaggerated immobility in the forced swim test compared to their control the Wistar rats (Paré, 1994, Nespor et al 2001). In addition, clinically effective antidepressants normalize these behaviors in WKY rats.

Groups of adult female WKY and Wistar rats (8/group) will be administered various doses (0.01, 0.02, 0.05, 0.1,and 0.5 mg/Kg) of estrogen (17-beta estradiol) or vehicle subcutaneously, and 30 min later they will be tested in elevated plus maze (5 min), locomotor

activity chamber (10 min) and Porsolt swim test (5 min). For chronic studies the same dose will be administered daily and animals will be tested on day 7, 14 and 21.

Total rats required = 48 (2groups x 8/group x 3 doses = 48)

Specific Aims 2.

Objective:

Determine whether depressive characteristics in an animal model of depression is associated with low levels of estrogen.

Protocol

Adult female WKY and Wistar rats (10/group) will be sacrificed by decapitation and plasma levels of estrogen (17-beta estradiol) will be determined by radioimmunoassay (RIA).

Total rats required = 20 (2 group x 10/group)

Specific Aims 3.

Objective:

Determine whether acute or chronic tamoxifen or raloxifene may result in depressive characteristics in an animal model.

Protocol

Groups of adult female WKY and Wistar rats (8/group) will be administered various doses (0.1, 0.5 and 1.0 mg/kg) of tamoxifen, raloxifene or vehicle intraperitoneally and 30 min later they will be tested in elevated plus maze (5 min), locomotor activity chamber (10 min) and Porsolt swim test (5 min). For chronic studies the same dose will be administered daily and animals will be tested on day 7, 14 and 21.

Total rats required = 128 (2groups x 8/group x 2 drugs x 4 doses = 128)

Specific Aims 4.

Objective:

Determine the effects of chronic estrogen, tamoxifen and raloxifene on biogenic amine neurotransmission in discrete brain regions.

Protocol

Microdialysis technique will be applied in evaluating the effects of 17-beta estradiol, tamoxifen and raloxifene on dopaminergic noradrenergic and serotonergic neurons in selective pathways. These pathways will include the shell region of nucleus accumbens (NACC) and the frontal cortex, terminal fields of mesolimbic and mesofrontal pathways, respectively. Although the release of dopamine in the NACC is of primary interest, the release of all three biogenic amines in the frontal cortex will be of significant relevance. This is because the frontal cortex also receives a significant noradrenergic input from the locus coeruleus and serotonergic input from the dorsal raphe.

Groups of untreated WKY and Wistar rats (8/group) will be implanted with microdialysis probes in nucleus accumbens (shell region) and the frontal cortex. Separate groups of rats will be used for each region. They will be administered estrogen, tamoxifen or raloxifene (the highest effective doses as determined in protocol 1 and 3) and the concentration of DA, NE and 5HT in the dialysate will be determined by HPLC-EC.

Number of rats required = 120 (8/group x 2 group x 2 areas x 3 treatments = 96, in addition, 24 rats will be required to determine the stereotaxic coordinates for each region in each strain: 2 strain x regions x 6 rat/region = 24).

ANIMALS

The experiment will be conducted using adult female WKY and Wistar rats. Upon arrival, the animals will be housed at the Veterinary Services, College of Medicine, and Howard University. The animals will be maintained in a group of three or four in a plastic cage at a temperature of 22^0 C and humidity (50 %), exposed to 12-hour light and 12 dark cycles with free access to food and water. The animals will be kept under quarantine for one week prior to

BEHAVIORAL EVALUATIONS

SWIM TEST

The swim test will be conducted in a cylindrical tank 60 cm tall and 18 cm diameter, containing enough 25^o C water so that the rat could not touch the bottom with its hindpaws. The animals will be placed in the water and the amount of time it is immobile will be recorded over a single 5 min period (Tizabi et al 1999b, 2000b). It is of relevance to note that immobility of the FSL rats at 5 min correlated highly with immobility at 10 min (Overstreet et al 1994). Therefore, the shorter session will be used in this study. Moreover, it was observed that FSL rats are very immobile, so it was not necessary to have the 15 min pretest session, as is common in standard Porsolt test protocols for other rats (Overstreet et al 1995). The tests will be carried out during the early part of the dark phase, between 10.00 and 14.00 h (Tizabi et al 1999b, 2000b). The validity of this test as an index of depression is well established (Overstreet et al 1994, Pare' 1994, Lucki 1997, Weiss et al 1998). In addition to immobility and swimming time, climbing and diving activity will also be quantified according to Lucki et al (1997). Climbing effort is a reflection of active rather than passive (immobility) behavior and has been attributed to catecholaminergic functions (Lucki 1997, Page et al 1999, see also preliminary results above).

Thus, in our new procedure, the behavior of rats in the swim test will be videotaped. This will not only allow a more accurate scoring capability of all the parameters, but will also afford a collection of permanent record for future viewing if necessary.

LOCOMOTOR ACTIVITY TEST

This test will be conducted in an automated "open field" photocell cage as described in detail (Richardson and Tizabi 1994, Tizabi et al 1997).

ELEVATED PLUS- MAZE Briefly, spontaneous locomotor activity, determined by the total horizontal distance traveled as well as vertical activity determined by the number of rearing will be automatically gathered. Animals will be monitored continuously for 10 minutes. The activity-monitoring cage will be wiped with soap and water after each use. This test will be performed immediately prior to the swim test.

The elevated- plus maze is widely used to test anxiogenic or anxiolytic effects of drugs in rats. The test relies on the relative aversion of rats to walk onto narrow, open arms of the maze as compared to the region that is protected by walls (Pellow et al. 1985; Duncan et al. 1996). Rats make significantly fewer entries into the open arm and spend less time in the open arms as compared to the closed arms of the +maze. Agents that may cause anxiety are likely to reduce the percentage of entries into, and time spent on the open arms. Therefore, the entries made to onto the open arms and the time spend on the open arms are considered to be correlated with anxiety. Expression of the data as the percentage of the total number of arm entries (% number of open arm entries) or total time spent (% time on the open arms) on either the open or the closed arms will be used to correct for the overall changes in exploration of the maze technique (File, 1992 ; Liter1987). An elevated plus-maze with two open arms (23×6 cm) and two closed arms (23×6×15 cm) that extend from a central platform will be used .The platform is mounted on

a base that is 60 cm high from the floor. Video- camera will be setup and used to record the activities on the two closed and opened arms that extend from the central platform. The animal will be placed in the center of the maze and the following parameters will be scored: time spent in the middle of the central platform and frequency of crossing, time spent in the open arm, time spent in the closed arm, and total number of crossing between arms. The camera automatically records the variables and the data will be later processed using software. Each test will be conducted for five minutes for each animal and the apparatus will be cleaned after each test.

MICRODIALYSIS

Rats will be anesthetized with pentobarbital (60 mg/kg ip) and placed in a Kopf stereotaxic apparatus. After the skull is exposed, two burr holes will be drilled for stereotaxic implantation of two microdialysis probes using standard procedures (Yoshimoto and McBride 1992) with coordinates determined according to the atlas of Paxinos and Watson (1986). Loop style probes will be used as described (Perry and Fuller 1992) except that these probes will be secured in 18 gauge thin-wall stainless-steel wire, which allows for more accurate placement during surgery (Campbell and McBride 1995). The microdialysis probe will be implanted into areas of interest and will be secured with stainless-steel screws and fixed in place with cranioplastic cement. The animals will be allowed 48 hours to recover from surgery before initiating experiments. Surgery will be performed under aseptic condition. Experiments will be performed in awake, freely moving animals. A liquid swivel will be used to connect the microdialysis probes to the microinfusion pump. Artificial cerebral spinal fluid (CSF) (composition in mM: 145 NaCl, 2.7 KCl, 1.0 $MgCl_2$, 1.2 $CaCl_2$, pH 7.4 with NaH_2PO_4) will be filtered though a 0.2 um sterile filter and perfused through the probe at 0.5 ul/min for 60-90 min before baseline samples are collected. Baseline samples will be collected every 20 minutes for

120 minutes. Dialysates will be collected in vials containing 2 ul 0.2 N HCl and will be immediately frozen on dry ice and stored at -80^{O} C until analysis.

At the end of the experiment, a 1% solution of bromphenol blue in artificial CSF will be perfused through the probes to verify placements. Animals will be overdosed with CO_2, decapitated and the brains removed. Brains will be stores at -80° C. Frozen sections will be prepared and probe placements verified according to the atlas of Paxinos and Watson (1986). Only data from animals with verified placements will be analyzed.

MEASUREMENT OF BIOGENIC AMINES

Concentrations of dopamine (DA), norepinephrine (NE) and serotonin (5HT) in dialysates will be carried out by HPLC- electrochemical detectors (Campbell and McBride 1995, Campbell et al 1996, Yoshioka et al 2000, see also preliminary data). The pre-column and analytical column will be UniJet ODS, 3 um, 14mm x 1mm I.D., respectively. The columns will be maintained at 27.5° C using a LC-22C temperature controller. The mobile phase will consist of a mixture of 1000 mL buffer (50 mM sodium citrate, 25 mM monobasic sodium phosphate, 10 mM diethylamine hydorochloride, 2.2 mM sodium octylsulfonate and 0.03 mM disodium EDTA, pH 3.2, adjusted with 85% phosphoric acid), 30 mL acetonitrile, 15 mL dimethylacetamide, and will be filtered through a 0.2 um filter. The flow rate will be at 0.2 mL/min. The back pressure of the system will be 3000-3500 psi. Detection will be at a glassy carbon working electrode (3 mm) maintained at a potential of +700 mV vs Ag/AgCl.

DATA ANALYSIS

Applicable parametric statistical tests will be used to analyze the data. Analysis of the data from the proposed experiments will usually require analysis of variance (ANOVA), since most experiments use more than two groups and/or multi-factor designs. When significant

differences are obtained with ANOVAs, Newman-Keuls post hoc test will be applied to conduct individual comparisons between groups. In the event that comparisons between only two groups are needed, appropriate t-tests will be used to assess significant differences. All analyses will utilize two-tailed distributions and significance level of 0.05.

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SPECIFIC AIMS OF RESEARCH INTERESTS

The development of cisplatin resistance by the cancer cell is one of the major drawbacks in treating patients with cisplatin chemotherapy. Although cisplatin resistance is multifactor, the defective accumulation of cisplatin has emerged as a prominent feature in many cisplatin-resistant cell lines. It is known that the cytotoxicity of cisplatin is dependent on the amount of drug accumulated in the cell. It is also known that a wide variety of physiological and pharmacologic manipulations can modulate the cellular accumulation of cisplatin. For example, cisplatin accumulation is partially dependent on the extracellular Na^+ concentration, and is altered by osmotic strength, pH, membrane polarization, protein kinase C agonists, calmodulin antagonists, calcium channel blockers, ATP and cAMP. The mechanism by which cisplatin accumulation is reduced in cisplatin-resistant cells is not known. The lack of such knowledge hinders the rational design of specific strategies to overcome accumulation-mediated resistance.

Our ***long-term goal*** is to circumvent cisplatin resistance in cells having the cisplatin accumulation defect. The ***objective of our research***, which is the next step in the pursuit of that goal, is to evaluate the relative contribution of cisplatin accumulation to the cytotoxic activity of cisplatin. The ***central hypothesis*** is that a specific terbium/cisplatin binding protein plays a key role in the transport of cisplatin across the plasma membrane. This theory has been formulated on the basis of preliminary data produced in our laboratory. Terbium, a lanthanide metal, was found to increase the cellular accumulation and cytotoxicity of cisplatin in human breast cancer cells. The combination of cisplatin and terbium was more effective in cisplatin-resistant cells than in cisplatin-sensitive cells. We discovered the terbium/cisplatin binding protein by using time-resolved terbium luminescence. In our most recent preliminary studies, gadolinium was also found to increase the cellular accumulation of cisplatin in human breast cancer cells. The ***rationale*** for this research is that, once the factors that influence the cellular accumulation of cisplatin are known, they can be used to enhance the antitumor efficacy of cisplatin. Our laboratory is unique in its capacity to conduct cancer research from a biophysical point of view at a Historically Black College or University. In addition, we are particularly well prepared to undertake this research, because we have already developed and validated the instruments that will be used to conduct the *in vitro* studies. The principal investigator is a trained Ph.D. Biophysicist with over twenty (20) years of experience in biophysical cytochemistry. He was the first to establish that the membrane binding of cisplatin is to a specific terbium/cisplatin binding protein. This work will be conducted in a research environment that is conducive for its successful completion. In addition, the Director of the Cancer Center has expressed her enthusiastic support for this project.

We propose to test our central hypothesis and, thereby, achieve the objective of this research, by pursuing the following ***three specific aims***:

1. **Evaluate the effects of gadolinium on the cellular accumulation and cytotoxicity of cisplatin in cisplatin-sensitive and cisplatin-resistant human breast cancer cells *in vitro*.**

The working hypothesis for this aim is that the cellular accumulation of cisplatin will increase in the presence of gadolinium, thus increasing the cytotoxicity of cisplatin. This is based on our preliminary studies. Further, gadolinium is a lanthanide metal, having one proton less than terbium. The chemistry and physiological nature of gadolinium are very similar to that of terbium.

2. **Evaluate the combined effects of cisplatin and gadolinium on the growth of cisplatin in cisplatin-sensitive and cisplatin-resistant human breast cancer transplants *in mice*.**
The working hypothesis for this aim is that the growth of human breast cancer transplants will decrease to a greater extent in the presence of cisplatin combined with gadolinium than in the presence of cisplatin alone. This is based on our preliminary studies, and on the concept that the pharmacologic behavior of drugs *in vivo* will be similar to their behavior *in vitro*.

3. **Determine that the membrane binding of gadolinium is to the terbium/cisplatin binding protein *in vitro*.**
Based on our preliminary data, the working hypothesis for this aim is that gadolinium will competitively decrease the membrane binding of terbium, without affecting the binding of cisplatin.

Various strategies have been developed to address specific features of cisplatin resistance. Since cisplatin accumulation is a major determinant of its antitumor activity, research into the mechanism of cisplatin transport is warranted. The proposed research represents an ***innovative*** approach to cancer research and treatment. It capitalizes on a novel method to increase the cytotoxicity of cisplatin. Our ***expectation*** is that, at the conclusion of this research, we will have confirmed that the coadministration of cisplatin and gadolinium will effectively eliminate cisplatin-resistant cells. We expect that the combination of cisplatin and gadolinium will permit the treatment of breast cancer patient with lower doses of cisplatin. Increasing our basic understanding of the interactions of cisplatin with the terbium/cisplatin binding protein will provide important insight on how breast cancer cells become resistant to cisplatin and on how to circumvent cisplatin resistance. Complete characterization of the terbium/cisplatin binding protein may facilitate the development of cancer-specific drugs devoid of unwanted side effects. Such an outcome is expected to have substantive ***impact*** on the future abilities of physicians to treat cancer patients.

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RESEARCH DESIGN AND METHOD

The primary objective of our research is to determine whether gadolinium can increase the antitumor activity of cisplatin in athymic nude mice. We plan to examine four different human breast cancer cell lines: MDA, MDA/CH, MCF-7 and MCF-7/CH. Each cell line was chosen because of their hormone dependency, cisplatin sensitivity and mechanism of cisplatin resistance. The main mode of cisplatin resistance in MDA cells is by elevated levels of glutathione, while cisplatin-resistant MCF-7 cells have exhibited a defective accumulation of the drug. The cisplatin-sensitive MDA cells are estrogen receptor negative, hormone-independent cells, and will be used as our experimental control line. In a small pilot project, gadolinium was found to increase the cellular accumulation and cytotoxicity of cisplatin in the MDA cell lines. The cisplatin-sensitive MCF-7 cells are estrogen receptor positive, hormone-dependent cells.

The importance of gadolinium-based magnetic resonance imaging in cancer treatment and research is unquestioned. We will use gadolinium chloride (Gd-Cl) and gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) in these studies. Chelation of gadolinium with DTPA reduces its toxicity. However, the physicochemical characteristics of Gd-Cl may increase the membrane interaction of gadolinium. Consequently, we plan to use both Gd-Cl and Gd-DTPA. Further, the toxicity of gadolinium is reduced at low concentrations of Gd-Cl.

SPECIFIC AIM: **Evaluate the combined effects of cisplatin and gadolinium on the growth of human breast cancer transplants *in mice*.**

The working hypothesis for this aim is that the growth of human breast cancer transplants will decrease to a greater extent in the presence of cisplatin combined with gadolinium than in the presence of cisplatin alone. This is based on our preliminary studies, and on the concept that the pharmacologic behavior of drugs *in vivo* will be similar to their behavior *in vitro*.

It is not known whether the preliminary results obtained ***in vitro*** will mirror those ***in vivo***. Therefore, research must be performed on animals to obtain any meaningful results applicable to humans. In the experiments describe here, we will use an athymic nude mouse model to study the effects of cisplatin and/or gadolinium (i.e., Gd-Cl and Gd-DTPA) on the growth of subcutaneous transplants of cisplatin-sensitive and cisplatin-resistant human breast cancer cells via bioluminescence imaging.

Healthy 7-week old female athymic nude mice (18-22 g) will be randomly assigned to each of three groups (12 mice/group), a tumor-free control and two tumor-bearing experimental groups. Animals will be housed in individual cages, at room temperature (22-25°C) and controlled in 12-hour light-dark diurnal cycles. Experimental animals will be subjected to subcutaneous

transplantation of **1.)** cisplatin-sensitive or **2.)** cisplatin-resistant dissociated cells from human breast cultures. Cancer cell inoculum will be assigned randomly across all tumor-bearing control and experimental groups. Subcutaneous transplantation will be performed under sterile conditions. The cancer cell inoculum will be loaded into trocars for subcutaneous inoculation in the back of the animal at a volume of 0.2 ml, a volume expected to contain approx. 10^5 cells. Prior to these experiments, standard curves will be constructed for each cell line. The mean number of photon will be measured as a function of cells (25 to 2.5 x 10^5) transplanted into the animals.

Experimental animals in groups 1 and 2 will be inoculated with tumor tissue and those in group **3** will be tumor-free controls. Animals will be age-matched and fed a diet containing normal levels of all dietary nutrients.

Food and drinking water will be provided *ad libitum*. Because of the sensitivity of tumor growth to food intake, food intake will be carefully measured three times per week, accounting for spillage, and daily intake values will be computed. Usually, during a 28-day experiment, 10%-30% of the experimental animals might expire. Their data will be omitted; thereby, having the potential to decrease the numbers in each of the experimental tumor-bearing groups to, at worst case, 10 mice. Twelve animals will be used in each control and experimental group, therefore, to obviate the possibility that too small a number of surviving experimental animals could compromise statistical significance of these studies. Death will not be used as an endpoint in this research. Body weight-loss is a reliable indicator for the time of death in tumor-bearing animals. The signs of pain and distress are readily recognized and assessed in laboratory rodents. Tumors will be considered painful, when the animals display abnormal locomotion, cease to eat and drink normally, bite or mutilate their affected area; at which time, the animals will be sacrificed via cervical dislocation. Mice will be sacrificed when their weights decrease by 10% of their original value.

Growth Modulation of Cisplatin-Sensitive and Cisplatin-Resistant Cancer Cells by Gd-Cl and Gd-DTPA: Twelve animals in the control and twelve animals in each of the experimental groups will be intravenously injected (0.1 ml, via the tail vein) with a vehicle (control), cisplatin, Gd-Cl, Gd-DTPA, cisplatin + Gd-Cl, cisplatin + Gd-DTPA on days 2, 5 and 8 after cancer cell transplantation. Initially, (unless determine otherwise), the following drug concentrations will be used in all cases: cisplatin = 3 mg/kg, Gd-Cl = 37.2 mg/kg and Gd-DTPA = 59 mg/kg. Studies will be performed on animals with cisplatin-sensitive and cisplatin-resistant tumor transplants of human breast cancer cells using the same methods as described above for characterizing the growth of these tumor transplants. The data will be evaluated by descriptive statistics, Student's *t*-test, one-way ANOVA, as well as presented in graphical or tabular format to illustrate the effects of drug combination or time after drug treatment on cancer cell number (i.e., Mean Photon Counts). Using a two-way ANOVA, the data obtained for the control cisplatin-sensitive parent cell line will be compared to the data for each cisplatin-resistant daughter cell line, as well as that of the other cell lines.

GRADUATE ONCOLOGY COURSE
SYLLABUS

EPIDEMIOLOGY OF CANCER

Lucile Adams-Campbell, Ph.D., Director HUCC & Professor of Medicine
Flora Ukoli, MD; D.P.H., MPH, Pamela Carter-Nolan, Ph.D., Division of Epidemiology and Biostatistics, HUCC

Educational Objectives:

1. To provide an understanding of epidemiology in cancer research.
2. To provide an understanding of the epidemiology of specific cancers which effect minority communities (e.g., breast, prostate, GI, etc.).
3. To evaluate the cause-effect relationships that may exist between risk factors and specific cancers effecting minority populations (e.g., evaluating the consistency of epidemiologic data with etiologic hypotheses identified either clinically or experimentally)..
4. To provide the basis for developing and understanding preventive procedures and public health practice.

Instructional Units/Topics:

1. *Basic Concepts*
   - Causation and Causal Inference
   - Molecular Epidemiology in Cancer Prevention
2. *Magnitude of Cancer*
   - Cancer Incidence, Mortality, and Survival among Racial and Ethnic Minority Groups in the US
3. *Causes of Cancer*
   - Risk factors associated with cancers that effect Minority Populations
4. *Cancer Prevention and Control*
   - Principles and Applications of Cancer Prevention
   - Health Education and Health Promotion
   - Clinical Trials
   - Fundamental Issues in Screening

References: *(Tentative)*

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2. American Cancer Society. 1998. Cancer Facts and Figures -- 1998. Atlanta, American Cancer Society.
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4. Baquet CR, Horm JW, Gibbs T, et al. 1991. Socioeconomic factors and cancer incidence among blacks and whites. J Natl Cancer Inst 83:551-557.
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CANCER STATISTICS AND STATISTICAL METHODS; EPIDEMIOLOGY OF CANCER

Kyung Sook Kim, Ph.D., assistant professor, Division of Epidemiology and Biostatistics, HUCC

Introduction to Biostatistics

Kyung Sook Kim, Ph.D., Assistant professor, Department of Community Health and Family Practice.

Educational Objectives:

Upon successful completion of this component, participants will be able to

1. Develop an understanding of the statistical concepts and of how and when to apply various statistical techniques.
2. Better understand published medical literature and critically evaluate authors' conclusions.

Instructional Units:

The student shall distinguish the following concepts:

1. Descriptive statistics/ Graphic representation
2. Probability
3. Normal Distribution
4. Statistical Inference
5. Correlation, Regression
6. Survival Curve
7. Sample size, power

References:

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CELL CYCLE AND CANCER

Hassan Ashktorab, Ph.D., *graduate assistant professor*, GI Division, Department of Medicine, Howard University; Gastrointestinal Carcinogenesis Laboratory, Howard University Cancer Center.

Instructional Units/Topics

1. Biochemistry and genetics of the cell cycle; growth factors, growth factor receptors, and receptor-mediated signaling; cell cycle regulators (cyclins and cyclin-dependent kinases (CDKs); inhibitors of CDKs (CDIs)
2. Cell cycle-related gene expression, checkpoints, transitions.
3. Cell cycle dysregulation and oncogenesis
4. p53, Rb, etc tumor suppressors and regulation of cell cycle

MOLECULAR DIAGNOSIS OF CANCER

Hassan Ashktorab, Ph.D., *graduate assistant professor*, GI Division, Department of Medicine, Howard University; Gastrointestinal Carcinogenesis Laboratory, Howard University Cancer Center.

Instructional Units/Topics

1. Current methods for molecular diagnosis of various cancers
2. Molecular basis of diagnostic techniques for breast cancer
3. Translational studies and clinical benefits

References:

Cell Cycle and Cancer

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Cell cycle machinery

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NUTRITION AND CANCER

Tanya Agurs-Collins, Ph.D., R.D., Nutrition Epidemiologist, Howard University Cancer Center, *and Assistant Professor*, Department of Community Health and Family Practice, Howard University College of Medicine.

Educational Objectives:
Upon successful completion of this component, participants will be able to:

Describe the biological principles of nutritional oncology
Understand the mechanisms/pathways that link nutritional status and the etiology of cancer

Understand the relationship between diet, nutrition and cancer prevention.

Instructional Units/Topics:

1. Fundamentals of nutrition: applications to cancer research
2. Epidemiology basis of nutritional influences on cancer
3. Dietary assessment and cancer prevention
4. Fruits and vegetable intake and cancer prevention
5. Energy balance, anthropometry and cancer
6. Dietary fiber, carbohydrate and cancer
7. Dietary lipid, alcohol and cancer
8. Functional foods and cancer prevention

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CANCER CHEMOTHERAPY: AGENTS AND TARGETS

Fred Lombardo, Pharm. D., College of Pharmacy and Pharmacal Sciences, and H.U. Cancer Center Faculty.

Topics/instructional units

Agents utilized in cancer therapy

Cell cycle specificity: cytotoxicity

S-phase (antimetabolites)

1. Antifolates (Methotrexate, Trimetrexate)
2. Antipyrimidines (Cytarabine, 5-Fluorouracil, etc)
3. Antipurines (6-Mercaptopurine, 6-Thioguanine)
4. Miscellaneous Agents (Hydroxyurea, Procarbazine)

G-2 Phase

Bleomycin

M-Phase

1. Vinca alkaloids (Vincristine, Vinblastine, Vinorelbine)
2. Podophylotoxins (Etoposide, Teniposide)
3. Taxanes (Taxol, Taxotere)

G-0 Phase

1. Nitrosoureas
2. Alkylators
3. Intercalators

G-1 Phase

1. L-asparaginase
2. Steroids

Cell cycle Non-Specificity

Apoptosis

Anti-Angiogenic Agents

Monoclonal Antibodies in cancer therapy

Cytokines/Chemokines

APOPTOSIS AND CANCER

Theodore A. Bremner, Ph.D., *graduate associate professor*, Department of Biology, and Tumor Biology Laboratory, Howard University Cancer Center; adjunct associate professor of molecular biology (research), Department of Cell Biology, Molecular Biology, and Biochemistry, Brown University.

Educational Objectives:

Upon successful completion of this component, participants will be able to

1. Describe apoptotic pathways and discuss the importance of apoptosis as a mechanism of tissue homeostasis, and its abrogation in cancer.
2. Discuss the role of the Bcl-2 family of proteins in modulating apoptosis.
3. Understand the loss of susceptibility to apoptosis as a mechanism of tumor promotion, and resistance to cancer chemotherapeutic drugs.
4. Describe the caspase activation cascade involved in Fas-mediated apoptosis; the mechanism of activation of caspases, and the cleavage of critical death substrates during programmed cell death.
5. Describe the role of the p53 tumor-suppressor gene in the induction of apoptosis in response to DNA damage or viral infection.
6. Describe the association between the p53 proline/arginine 72 polymorphism and risk of human papillomavirus-associated cervical cancer.
7. Discuss the mechanisms of action of oncolytic viruses on p53-negative tumors.
8. Describe laboratory methods of detection and quantitation of apoptosis.

Instructional Units:

1. Tissue homeostasis: proliferation and apoptosis. Survival/anti-apoptotic signaling: Akt/PKB and NF-κB in anti-apoptotic signaling.
2. Oncogenes and tumor suppressor genes that affect (primarily) cell cycling, apoptosis, or both (excluding *ras*, *p53*, and *Rb*, covered earlier): *fos*, *APC*, *DCC*, *PTEN*, *Cbl*, *MTS1*, *BRCA1*. Fos and gene silencing via methylation; the APC/β-catenin signaling pathway; PTEN and abrogation of survival signaling; DCC, a dependence receptor. Fearon-Vogelstein model of colorectal carcinogenesis.
2. Receptor-mediated apoptotic signaling: Fas/Apo-1/CD95 and Fas ligand (FasL) in immune regulation and cell-mediated killing.
3. Proapoptotic and antiapoptotic molecules: Bcl-2 family members, NF-κB, cIAP, superoxide.
4. Mechanisms of p53 potentiation of apoptosis in response to DNA damage and viral infection: ATM kinase and phosphorylation of p53; trancriptional regulation of *bax* and *bcl-2*; Fas trafficking.
5. Viral suppression of apoptosis: FLIPs, SV40 LTA, HPV-16 and HPV-18 E6 proteins and functional inactivation of p53.
6. Laboratory methods for the detection and quantitation of apoptosis: DNA laddering, nuclear morphology, phosphatidyl serine (PS) externalization and Annexin V binding; TUNEL analysis, flow cytometry (FCM) using propidium iodide, Hoechst 33342, Annexin V-FITC labeling. Interpretation of FCM data.

WWW RESOURCES

American Cancer Society: http://www.cancer.org/
Apoptosis Online: http://www.apopnet.com/
BioMedNet: http://www.BioMedNet.com
BioMedLink: http://biomedlink.com
Cancer Coalition: http://www.cancercoalition.org
Cell and Molecular Biology Online: http://www.cellbio.com/
Cells Alive: http://www.cellsalive.com
Howard Hughes Medical Institute: http://www.hhmi.org
Leukemia Society of America: http://www.leukemia.org/docs/fs_leuk_rel.html
National Library of Medicine (Internet Grateful Med; Pub Med; etc. Literature searches in all areas of the biomedical sciences): http://www.nlm.nih.gov/
National Cancer Institute (NCI): Cancer Trials; CancerNet™, NCI Event Calendar; Research at NCI; Office of International Affairs, etc.: http://www.nci.nih.gov

OncoLink (University of Peansylvania): http://oncolink.upenn.edu/upcc/
UICC (International Union Against Cancer/Union Internationale Contre le Cancer): http://www.uicc.org

Tutorials
Flow cytometry in cell cycle analysis and apoptosis: An excellent tutorial is available at the Derek Davies Home Page (Imperial Cancer Research Fund (ICRF) , London:

http://www.lif.icnet.uk/axp/facs/davies/annexin2.gif
Can be accessed through BioMedLink: http://biomedlink.com

About Apoptosis, by P. Henkart, NIH
A Brief Introduction to Apoptosis by L. W. Browder
Apoptosis, Radiosensitivity and the Cell Cycle, by W Gillies McKenna, M.D., Ph.D. in OncoLink
Can be accessed through http://www.apopnet.com/

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BIOLOGY OF RADIOTHERAPY

Raj Sridhar, Ph.D.,graduate associate professor, Dept. of Radiation Oncology, Howard University Hospital.

Topics/Instructional Units

1. Types of radiation used in cancer therapy
2. Interactions of radiation with matter
3. Concept of radiation
4. Dose-response curves, cellular target for radiation damage
5. Repair of radiation damage
6. Fractionation of radiation dose
7. Normal tissue tolerance and radiation response of tumors
8. Goals of radiation therapy and multi-modality treatment of cancer
9. Radiosensitization, radiopprotection and drug radiation interactions
10. Brachytherapy

References:

CHEMOPREVENTION AND CARCINOGENESIS

Joel Schwartz, D.M.D., D.M.Sc., Director of Research, College of Dentistry, Howard University

Department of Oral Maxillofacial Pathology, Rm. 2A-3, Laboratory, 2F-8. Pjones: (202) 806-0094; (202) 806-0345

Educational Objectives

Upon successful completion of this component, participants will have knowledge of:

1. The possible mechanisms for the prevention of cancer.
2. The interaction of nutrients with cellular processes.
3. The specific oxidative-redox features of nutrients that result in the modification of cellular processes.
4. The redox characteristics of tumor suppressor genes.
5. The redox features [describing features] of programmed cell death and DNA repair. (?)
6. Transcription response to redox molecules.
7. Alterations in the cell cycle as derivatives of chemopreventive actions.
8. Carcinogenesis described by molecular and histopathologic markers.
9. Molecular and histopathologic markers of chemopreventive function.

Topics/Instructional Units

1. Programmed cell death: the tumor suppressor and immune generated pathways: assays to assess chemopreventive modifications.
2. Laboratory methods to detect nutrient chemopreventive agents: animal and laboratory assays.
3. Laboratory methods to detect novel mutations in tumor suppressor genes and protocols to determine novel for early malignant transformation.
4. Focus on the interaction between nutrient chemopreventives, and/or diet and the process of oral carcinogenesis.

References:

CANCER GENETICS AND GENOMICS

Carolyn Whitfield Broome, Ph.D., Associate professor, Department of Biochemistry and Molecular Biology, College of Medicine, Howard University, and
Rick Kittles, Ph.D., Human Genome Research Associate, Cancer center, Howard University.

Educational Objectives

Instructional Units/Topics

References:

BEHAVIOR

Paige Green-McDonald, Ph.D., Assistant professor of Medicine, Division of Epidemiology and Biostatistics, Howard University Cancer Center.

Educational Objectives

Upon successful completion of this component, participants will be able to:

1. Describe the role of behavioral research in the prevention, early detection, and control of cancer
2. Identify and discuss priorities in behavioral research related to cancer prevention and control
3. Identify and describe health behavior theories in cancer prevention and control
4. Critically discuss and evaluate behavioral interventions used in cancer 0provention and control

Instructional Units/Topics

1. Risk factors: tobacco use, diet, physical exercise, alcohol use, stress, racism, socioeconomic status
2. Priorities in behavioral research in cancer prevention and control
3. Individual health behavior theories: Health Belief Model, Theory of Reasoned Action, Transtheoretical model of change, Prospect Theory, Transactional Model of Stress and Coping
4. Interpersonal health behavior theories: Social Cognitive Theory, social networks and social support, patient-provider communication
5. PRECEDE-PROCEED Planning Model
6. Race in cancer prevention and control
7. Behavioral intervention studies

References:

DRAFT LETTER TO DIRECTORS OF GRADUATE PROGRAMS AND CHAIRS OF DEPARTMENTS

Dear Director, chairman, etc.:

The Howard University Cancer Center would like to request your help in developing a graduate course in oncology. We also invite you to suggest ways of increasing the interest of graduate, medical and other students in such a course. At present, there is no comprehensive course in oncology at the university. The proposed course will utilize the primary literature, and instructional units will be designed to give the student a background in the processes associated with the development of cancer as well as to stimulate interest in cancer research.

The course will also cover prevention, detection and treatment of various cancers with special emphasis on those cancers that have a disparate impact on minority populations. We believe that the experience would be a valuable supplement to graduate education in a variety of biomedical specialties. The teaching staff will be drawn from the various colleges of the university, but primarily from faculty conducting ongoing basic and clinical research at Howard University Cancer Center. In addition, noted authorities in various specialties will be invited as guest lecturers. They will be selected for their excellence in cancer research and training, as well as for their interest in cancer in minority populations.

Please assist us by estimating the number of graduate students in your administrative area who might be interested in registering for this course. A copy of the draft syllabus is enclosed for your comments and suggestions which should be forwarded to the address below no later than _____.

Dr. Lucile Adams-Campbell, Director, HUCC
Chair, Graduate Oncology Course Committee
Howard University Cancer Center
2041 Georgia Avenue, NW
Washington, DC 20060
E-mail: ladams-campbell@fac.howard.edu
Telephone: (202) 806-7697; Fax: (202) 667-1686

We thank you for your assistance in this endeavor.

Sincerely

Lucile L. Campbell-Adams, Ph.D.
Professor

GRADUATE ONCOLOGY COURSE

Proposed Class: 12 to 15 for the first year, with projected growth of approximately 20% per year to a total of 30.

Class meetings, 2 meetings per week, 90 minutes each

Proposed Times 5:00 - 6:30 PM, Mon, Wed.

Tentative list of prospective guest lecturers and consultants

Donald Coffey
Marc Lipman
Richard Klausner
Nancy Dawson
Eddie Reid
Bruce Trock
John Finerty
Curt Harris
E. Premkumar Reddy

21 January 2000

Meeting with Dr. Donald Coffey
Brady Urological Institute (Rm 121)
Marburg Building
Johns Hopkins Medical Institutions

SUBJECT: Collaboration on developing the "Integrative Oncology Course" for the HU College of Medicine through the HU Cancer Center

Prospective JHU Faculty	Expertise
Stewart Grossman	Neuro-oncology and pain
Steve Piantidosi	Statistics/ Clinical trials
Bert Vogelstein	Cell cycle control
Ken Kensler	Cell cycle control
Ted De Weese	Radiation biology/radiotherapy
William G. Nelson	Nutrition and cancer, molecular biology, therapy
John Isaacs	Cancer and chemotherapy
Don Coffey	Apoptosis, chemotherapy, epigenetics, prevention
Tom Kensler	Chemoprevention
John Groopman	Chemoprevention
Jim Zabor	Behavior; cancer control
Scott Kearn	Cancer genetics (pancreas)
Steve Baylin	Methylation
Drew Pardoe	Cancer immunology
Hilivitski	Cancer immunology
Liz Jaffe	Cancer immunology
Jim Herman	Methylation

Opening lecture by Don Coffey:

An Overview to the Understanding and Control of Cancer

PROPOSED SCHEDULE OF LECTURES FOR THE FIRST YEAR
The class will meet twice weekly for 90 minutes per session.
Proposed hrs: Mon. 4:00 to 5:30 PM; Wed 4:00 to 5:30 PM

FIRST SEMESTER
Lectures 1 and 2, (lecture titles are tentative):

Week 1 *Introduction and welcome. Opening lecture.*
1. *An Overview to the Understanding and Control of Cancer.*
   Dr. Donald Coffey, Johns Hopkins Oncology Center (JHOC)
2. *Genetics and Pathology of Cancer: microarray technology and cancer diagnosis.*
   Dr. Donald Coffey, JHOC

Week 2 *Genetics of Cancer*
1. Dr. Kenneth Kinzler, JHOC
2. To be determined

Week 3 *Epigenetics of Cancer*
1. Dr. Theodore A. Bremner, Howard University Cancer Center (HUCC)
2. Dr. Stephen Baylin, JHOC

Week 4 *Cell Cycle and Cell Growth*
1. Dr. Hassan Ashktorab, HUCC
2. Dr. Chi Dang, JHOC

Submission of research proposal abstracts. 3 - 5-page concept paper.

Week 5 *Apoptosis*
1. Dr. Theodore A.Bremner, HUCC
2. Dr. John Isaacs, JHOC

Week 6 *Mechanisms of Carcinogenesis*
1. Dr. Joel Schwartz, College of Dentistry, Howard University
2. Dr. Thomas Kensler, JHOC

Week7 *Cancer Statistics And Statistical Methods*
1. Kyung Sook Kim, Ph.D., HUCC
2. Kyung Sook Kim, Ph.D., HUCC

Week 8 *Epidemiology of Cancer*
1. Dr. Pamela Carter-Nolan, HUCC
2. Dr. Flora Ukoli, HUCC

Week 9 *Cancer Prevention*

1. Dr. Joel Schwartz, Howard University College of Dentistry
2. Dr. John Groopman, JHOC

Week 10 *Radiation Therapy*

1. Dr. Raj Sridhar, HUCC
2. Dr. Theodore DeWeese, JHOC

Week 11 *Cancer Chemotherapy*

1. Dr. Frederick Lombardo, HUCC
2. Dr. William Nelson, JHOC

Week 12 *Cancer Immunology*

1. Dr. Drew Pardoll, JHOC
2. Dr. Hyam Levitsky, JHOC

Week 13 *Nutrition and Cancer*

1. Dr. Tanya Agurs-Collins, HUCC
2. Dr. William Nelson, JHOC

Week 14 *Behavior and Cancer*

1. Dr. Paige McDonald, HUCC
2. Dr. Richard Klausner (proposed, to be invited)

Week 15 *Oral and written presentations of research proposals:* Students will present their proposals for research in any of the areas covered in the course to a panel of HUCC and JHOC faculty. Reviewers will critique proposals for aims, feasibility, background, and methods.

Reading period Faculty will be available for conferences, discussions, etc.

FINAL EXAMINATION:

STUDENTS WILL TAKE A WRITTEN EXAMINATION IN ESSAY FORMAT DESIGNED TO TEST THEIR KNOWLEDGE AND APPLICATION OF SKILLS AND CONCEPTS DEEMED BY THE TEACHING FACULTY TO BE OF FUNDAMENTAL IMPORTANCE TO THE AIMS AND OBJECTIVES OF THE COURSE.

SECOND SEMESTER

Registration for the second semester will be limited to students whose performance in the first semester was superior and who demonstrated an active interest in cancer research. It is proposed that the second semester will be devoted to research seminars and focused discussions, to assist students in identifying specific areas of investigation and career opportunities. **The syllabus for the second semester will be developed in consultation with all members of the teaching staff and other consultants as needed.**

Topic areas to be developed include the following:

1. Principles of research and Experimental design
2. Exploration of gene and protein sequence databases in relation to cancer
3. Development of grant proposals for support of pre-doctoral and post-doctoral cancer research
4. Completion of NIH Form 398, and similar instruments
5. Applications for Internal Review Board (IRB) approvals, consent forms, etc.
6. Animal models (transgenic and orthotopic mouse models, etc)
7. Translational studies and clinical trials
8. Ethics and Scientific misconduct

GRADUATE ONCOLOGY COURSE
SYLLABUS

CANCER GENETICS AND ONCOGENES

Carolyn Whitfield Broome, Ph.D., graduate associate professor, Department of Biochemistry and Molecular Biology, College of Medicine,Howard University, and Howard University Cancer Center.

Educational Objectives

1. To understand the role of germ line and somatic cell mutations in the development of cancer, particularly in African Americans.
2. To critically evaluate the literature
3. To understand the methods used to detect mutations.
4. To examine the function of the normal and mutated gene.

Instructional Units/Topics

1. Breast Cancer
2. Prostate Cancer
3. Colorectal Cancer

References:

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carcinomas, and association of codon-12 valine with early mortality. J. Path. 185, 130-138.
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EPIGENETICS OF CANCER

Theodore A. Bremner, Ph.D., *graduate associate professor*, Department of Biology, and Tumor Biology Laboratory, Howard University Cancer Center

Educational Objectives

1. To explore the mechanisms of gene silencing by DNA methylation
2. To understand the mechanisms by which tumor suppressor genes can be silenced by DNA methylation.
3. To study the role of DNA methylation in the action of selected oncogenes.

Instructional Units

1. Regulation of transcription by modulation of chromatin architecture: histone deacetylase and DNA methyltransferase.
2. DNA methylation and the mechanism of *fos* tumorigenesis.

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1 Bakin, A. V., and Curran, T. (1999). Role of DNA 5-methylcytosine transferase in cell transformation by *fos*. *Science* **283**, 387-390.
2. Barletta, J.M., Rainer, S., and Feinberg, A.P. (1997). Reversal of loss of imprinting in tumor cells by 5-aza-2'-deoxycytidine. *Cancer Res.* **57**, 48-50.
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CELL CYCLE AND CANCER

Hassan Ashktorab, Ph.D., *graduate assistant professor*, GI Division, Department of Medicine, Howard University; Gastrointestinal Carcinogenesis Laboratory, Howard University Cancer Center.

Instructional Units/Topics

1. Biochemistry and genetics of the cell cycle; growth factors, growth factor receptors and receptor-mediated signaling; cell cycle regulators (cyclins and cyclin-dependent kinases (CDKs); inhibitors of CDKs (CDIs)
2. Cell cycle-related gene expression, checkpoints, transitions.
3. Cell cycle dysregulation and oncogenesis
4. p53, Rb, etc tumor suppressors and regulation of cell cycle

MOLECULAR DIAGNOSIS OF CANCER

Hassan Ashktorab, Ph.D., *graduate assistant professor*, GI Division, Department of Medicine, Howard University; Gastrointestinal Carcinogenesis Laboratory, Howard University Cancer Center.

Instructional Units/Topics

1. Current methods for molecular diagnosis of various cancers
2. Molecular basis of diagnostic techniques for breast cancer
3. Translational studies and clinical benefits

References:

Cell Cycle and Cancer

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Cell cycle machinery

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APOPTOSIS AND CANCER

Theodore A. Bremner, Ph.D., *graduate associate professor*, Department of Biology, and Tumor Biology Laboratory, Howard University Cancer Center

Educational Objectives:

Upon successful completion of this component, participants will be able to

1. Describe apoptotic pathways and discuss the importance of apoptosis as a mechanism of tissue homeostasis, and its abrogation in cancer.
2. Discuss the role of the Bcl-2 family of proteins in modulating apoptosis.
3. Understand the loss of susceptibility to apoptosis as a mechanism of tumor promotion, and resistance to cancer chemotherapeutic drugs.
4. Describe the caspase activation cascade involved in Fas-mediated apoptosis; the mechanism of activation of caspases, and the cleavage of critical death substrates during programmed cell death.
5. Describe the role of the p53 tumor-suppressor gene in the induction of apoptosis in response to DNA damage or viral infection.
6. Describe the association between the p53 proline/arginine 72 polymorphism and risk of human papillomavirus-associated cervical cancer.
7. Discuss the mechanisms of action of oncolytic viruses on p53-negative tumors.
8. Describe laboratory methods of detection and quantitation of apoptosis.

Instructional Units:

1. Tissue homeostasis: proliferation and apoptosis. Survival/anti-apoptotic signaling: Akt/PKB and NF-κB in anti-apoptotic signaling.

2. Oncogenes and tumor suppressor genes that affect (primarily) cell cycling, apoptosis, or both (excluding *ras*, *p53*, and *Rb*, covered earlier): *fos*, *APC*, *DCC*, *PTEN*, *Cbl*, *MTS1*, *BRCA1*. Fos and gene silencing via methylation; the APC/β-catenin signaling pathway; PTEN and abrogation of survival signaling; DCC, a dependence receptor. Fearon-Vogelstein model of colorectal carcinogenesis.
3. Receptor-mediated apoptotic signaling: Fas/Apo-1/CD95 and Fas ligand (FasL) in immune regulation and cell-mediated killing.
4. Proapoptotic and antiapoptotic molecules: Bcl-2 family members, NF-κB, cIAP, superoxide.
5. Mechanisms of p53 potentiation of apoptosis in response to DNA damage and viral infection: ATM kinase and phosphorylation of p53; trancriptional regulation of *bax* and *bcl-2*; Fas trafficking.
6. Viral suppression of apoptosis: FLIPs, SV40 LTA, HPV-16 and HPV-18 E6 proteins and functional inactivation of p53.
7. Laboratory methods for the detection and quantitation of apoptosis: DNA laddering, nuclear morphology, phosphatidyl serine (PS) externalization and Annexin V binding; TUNEL analysis, flow cytometry (FCM) using propidium iodide, Hoechst 33342, Annexin V-FITC labeling. Interpretation of FCM data.

WWW RESOURCES

American Cancer Society: http://www.cancer.org/
Apoptosis Online: http://www.apopnet.com/
BioMedNet: http://www.BioMedNet.com
BioMedLink: http://biomedlink.com
Cancer Coalition: http://www.cancercoalition.org
Cell and Molecular Biology Online: http://www.cellbio.com/
Cells Alive: http://www.cellsalive.com
Howard Hughes Medical Institute: http://www.hhmi.org
Leukemia Society of America: http://www.leukemia.org/docs/fs_leuk_rel.html
National Library of Medicine (Internet Grateful Med; Pub Med; etc. Literature searches in all areas of the biomedical sciences): http://www.nlm.nih.gov/
National Cancer Institute (NCI): Cancer Trials; CancerNet™, NCI Event Calendar; Research at NCI; Office of International Affairs, etc.: http://www.nci.nih.gov
OncoLink (University of Pennsylvania): http://oncolink.upenn.edu/upcc/
UICC (International Union Against Cancer/Union Internationale Contre le Cancer): http://www.uicc.org

Tutorials
Flow cytometry in cell cycle analysis and apoptosis: An excellent tutorial is available at the Derek Davies Home Page (Imperial Cancer Research Fund (ICRF) , London:

http://www.lif.icnet.uk/axp/facs/davies/annexin2.gif
Can be accessed through BioMedLink: http://biomedlink.com

About Apoptosis, by P. Henkart, NIH
A Brief Introduction to Apoptosis by L. W. Browder

Apoptosis, Radiosensitivity and the Cell Cycle, by W Gillies McKenna, M.D., Ph.D. in OncoLink
Can be accessed through http://www.apopnet.com/

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CARCINOGENESIS AND CHEMOPREVENTION

Joel Schwartz, D.M.D., D.M.Sc., Director of Research, College of Dentistry, Howard University

Department of Oral Maxillofacial Pathology, Rm. 2A-3, Laboratory, 2F-8. Pjones: (202) 806-0094; (202) 806-0345

Educational Objectives

Upon successful completion of this component, participants will have knowledge of:

1. The possible mechanisms for the prevention of cancer.
2. The interaction of nutrients with cellular processes.
3. The specific oxidative-redox features of nutrients that result in the modification of cellular processes.
4. The redox characteristics of tumor suppressor genes.
5. The redox features [describing features] of programmed cell death and DNA repair. (?)
6. Transcription response to redox molecules.
7. Alterations in the cell cycle as derivatives of chemopreventive actions.
8. Carcinogenesis described by molecular and histopathologic markers.
9. Molecular and histopathologic markers of chemopreventive function.

Topics/Instructional Units

1. Programmed cell death: the tumor suppressor and immune generated pathways: assays to assess chemopreventive modifications.
2. Laboratory methods to detect nutrient chemopreventive agents: animal and laboratory assays.
3. Laboratory methods to detect novel mutations in tumor suppressor genes and protocols to determine novel for early malignant transformation.
4. Focus on the interaction between nutrient chemopreventives, and/or diet and the process of oral carcinogenesis.

References:

EPIDEMIOLOGY OF CANCER

Lucile Adams-Campbell, Ph.D., Director HUCC & Professor of Medicine
Flora Ukoli, MD, D.P.H., MPH, Pamela Carter-Nolan, Ph.D., Division of Epidemiology and Biostatistics, HUCC

Educational Objectives:

1. To provide an understanding of epidemiology in cancer research.
2. To provide an understanding of the epidemiology of specific cancers which effect minority communities (e.g., breast, prostate, GI, etc.).
3. To evaluate the cause-effect relationships that may exist between risk factors and specific cancers effecting minority populations (e.g., evaluating the consistency of epidemiologic data with etiologic hypotheses identified either clinically or experimentally)..
4. To provide the basis for developing and understanding preventive procedures and public health practice.

Instructional Units/Topics:

2. *Basic Concepts*
   Causation and Causal Inference
   Molecular Epidemiology in Cancer Prevention
3. *Magnitude of Cancer*
   Cancer Incidence, Mortality, and Survival among Racial and Ethnic Minority Groups in the US
4. *Causes of Cancer*
   Risk factors associated with cancers that effect Minority Populations
5. *Cancer Prevention and Control*
   Principles and Applications of Cancer Prevention
   Health Education and Health Promotion
   Clinical Trials
   Fundamental Issues in Screening

References: *(Tentative)*

1. American Cancer Society. 1998. Cancer Statistics – 1998. CA Cancer J Clin. 48(1).
2. American Cancer Society. 1998. Cancer Facts and Figures -- 1998. Atlanta, American Cancer Society.
3. Austoker J. 1994. Cancer prevention in primary care. Current trends and some prospects for the future---II. BMJ 309:517-520.
4. Baquet CR, Horm JW, Gibbs T, et al. 1991. Socioeconomic factors and cancer incidence among blacks and whites. J Natl Cancer Inst 83:551-557.
5. Boring CC, Squires TS, Health CW Jr., et al. 1992. Cancer statistics for African-Americans. CA Cancer J Clin. 42:7-17.
6. Devesa SS, Blot WJ, Stone BJ, et al. 1995. Recent cancer trends in the United States.

J Natl Cancer Inst. 87:175-182.
7. Gorey KM, Vena JE. 1994. Cancer differentials among US blacks and whites: quantitative estimates of socioeconomic-related risks. J Natl Med Assoc 86:209-215.
8. Hill AB. 1965. The environment and disease: association or causation? Proc Soc Med 58:295-300.
9. Hulka BS. 1991. Epidemiological studies using biological markers: Issues for epidemiologists. Cancer Epidemiol Biomarkers Prev 1:13-19.
10. Jones PA, Buckley JD, Henderson BE, Ross RK, Pike MC. 1991. From gene to carcinogen: A rapidly evolving field in molecular epidemiology. Cancer Res 51:3617-3620.
11. Lacey L. 1993. Cancer prevention an early detection strategies for reaching underserved urban, low-income black women. Barriers and objectives. Cancer 72:1078-1083.
12. Landrigan PJ. 1992. Commentary: Environmental disease -- A preventable epidemic. Am J Public Health 82:941-943.
13. Li FP. 1990. Familial cancer syndromes and clusters. Curr Probl Cancer 49:75-113.
14. Olden K. 1994. Mutagen hypersensitivity as a biomarker of genetic predisposition to carcinogenesis. J Natl Cancer Inst 86:1660-1661.
15. Otten MW, Teutsch SM, Williamson DF, et al. 1990. The effect of known risk factors on the excess mortality of black adults in the United States. JAMA 263:845-850.
16. Renton A. 1994. Epidemiology and causation: a realist view. J Epidemiol Community Health 48:79-85.
17. Rothman KJ. 1976. Causes. Am J Epidemiol 104:587-592.

CANCER STATISTICS AND STATISTICAL METHODS; EPIDEMIOLOGY OF CANCER

Kyung Sook Kim, Ph.D., assistant professor, Division of Epidemiology and Biostatistics, HUCC

Introduction to Biostatistics

Kyung Sook Kim, Ph.D., Assistant professor, Department of Community Health and Family Practice.

Educational Objectives:

Upon successful completion of this component, participants will be able to

1. Develop an understanding of the statistical concepts and of how and when to apply various statistical techniques.
2. Better understand published medical literature and critically evaluate authors' conclusions.

Instructional Units:

The student shall distinguish the following concepts:

1. Descriptive statistics/ Graphic representation
2. Probability
3. Normal Distribution

4. Statistical Inference
5. Correlation, Regression
6. Survival Curve
7. Sample size, power

References:

1. Kuzma, Jan W., Basic Statistics for the Health Sciences, 2nd ed., Mayfield Publishing Co., Ca., 1992.
2. Johnson, R., Elementary Statistics, 4th ed., North Scituate, Mass: Duxbury Press.

   Milton, S., Statistical Methods in the Biological and Health Sciences, 3rd ed., WCB/McGraw-Hill.
3. Essex-Sorlie, D., Medical Biostatistics & Epidemiology, APPLETON & LANGE, 1995.
4. Lee, E., Statistical Methods for Survival Data Analysis, 2nd ed., John Wiley & Sons, Inc., 1992.

BIOLOGY OF RADIOTHERAPY

Raj Sridhar, Ph.D.,graduate associate professor, Dept. of Radiation Oncology, Howard University Hospital.

Topics/Instructional Units

1. Types of radiation used in cancer therapy
2. Interactions of radiation with matter
3. Concept of radiation
4. Dose-response curves, cellular target for radiation damage
5. Repair of radiation damage
6. Fractionation of radiation dose
7. Normal tissue tolerance and radiation response of tumors
8. Goals of radiation therapy and multi-modality treatment of cancer
9. Radiosensitization, radiopprotection and drug radiation interactions
10. Brachytherapy

References:

CANCER CHEMOTHERAPY: AGENTS AND TARGETS

Frederick Lombardo, Pharm. D., College of Pharmacy and Pharmacal Sciences, and H.U. Cancer Center Faculty.

Topics/instructional units

Agents utilized in cancer therapy
Cell cycle specificity: cytotoxicity

<u>S-phase (antimetabolites)</u>

1. Antifolates (Methotrexate, Trimetrexate)
2. Antipyrimidines (Cytarabine, 5-Fluorouracil, etc)
3. Antipurines (6-Mercaptopurine, 6-Thioguanine)

4. Miscellaneous Agents (Hydroxyurea, Procarbazine)

G-2 Phase
Bleomycin

M-Phase
1. Vinca alkaloids (Vincristine, Vinblastine, Vinorelbine)
2. Podophylotoxins (Etoposide, Teniposide)
3. Taxanes (Taxol, Taxotere)

G-0 Phase
1. Nitrosoureas
2. Alkylators
3. Intercalators

G-1 Phase
1. L-asparaginase
2. Steroids

Cell cycle Non-Specificity
Apoptosis
Anti-Angiogenic Agents
Monoclonal Antibodies in cancer therapy
Cytokines/Chemokines

NUTRITION AND CANCER

Tanya Agurs-Collins, Ph.D., R.D., Nutrition Epidemiologist, Howard University Cancer Center, *and Assistant Professor*, Department of Community Health and Family Practice, Howard University College of Medicine.

Educational Objectives:

Upon successful completion of this component, participants will be able to:

Describe the biological principles of nutritional oncology

Understand the mechanisms/pathways that link nutritional status and the etiology of cancer

Understand the relationship between diet, nutrition and cancer prevention.

Instructional Units/Topics:

8. Fundamentals of nutrition: applications to cancer research
9. Epidemiology basis of nutritional influences on cancer
10. Dietary assessment and cancer prevention
11. Fruits and vegetable intake and cancer prevention
12. Energy balance, anthropometry and cancer
13. Dietary fiber, carbohydrate and cancer
14. Dietary lipid, alcohol and cancer
15. Functional foods and cancer prevention

References:

1. Nutritional Oncology, Edited by: Heber D, Blackburn G, Go VLW, Harcourt Brace & Company 1998.
2. Weisburger JH. Can cancer risk be altered by changing nutritional traditions? Cancer 1998 Oct 1;83(7):1278-81.
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4. Kreb-Smith SM. Progress in improving diet to reduce cancer risk. Cancer 1998 Oct 1;83(7):1425-32.
5. Singh PN and Fraser GE. Dietary Risk factors for colon cancer in a low-income population. Am J Epidemiol 1998 Oct 15;14(8):761-74.
6. Clinton SK and Giovannucci E. Diet, nutrition, and prostate cancer. Annu Rev Nutr 1998;18:413-40.
7. Caygill GP, Charlett A, Hill MJ. Relationship between the intake of high-fibre foods and energy and the risk of cancer of the large bowel and breast. Eur J Cancer Prev 1998 May;7 Suppl2:S11-7.
8. Skog KI, Johansson MA, Jagerstad MI. Carcinogenic heterocyclic amines in model systems and cook foods: a review on formation, occurrence and intake. Food Chem Toxicol 1998 Sept-oct;36(9-10):879-96.
9. Hakim I. Mediterranean diets and cancer prevention. Arch Intern Med 1998 Jun 8;158(11):1169-70.
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11. DeWys WD. Diet and cancer prevention:an overview. Semin Oncol 1983 Sep;10(3)255-6
12. Bingham S. Food components and mechanisms of interest in cancer and diet in relation to their measurement. Eur J Clin Nutr 1993 Oct;47 Suppl 2:S73-7.
13. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vena-Ribes J. Functional food science and defense against reactive oxidative species. Br J Nutr 1998 Aug; 80 Suppl 1:S77-112.
14. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998 Nov;56(11):317-33.
15. Miller M. Can reducing caloric intake also help reduce cancer? J Natl Cancer Inst 1998 Dec 2;90(23):1766-7.
16. Key T. Micronutrients and cancer aetiology: the epidemilogical evidence. Proc Nutr Soc 1994,53,605-614.
17. Block G, Patterson B, Subar A. Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992, 18, 1-29.
18. Kumanyika S. Racial and Ethnic Issues in Diet and Cancer Epidemiology. In Diet and Cancer:
19. Markers, prevention, and Treatment. Edited by Jacobs MM, Plenum Press, New York 1994, 59-70.
20. Byers T. Nutritional Risk Factors for Breast Cancer. Cancer 1994;74:288-95.
21. Milner J. Nonnutritive Components in Foods as Modifiers of the Cancer Process. In: Preventive
22. Nutrition: The comprehensive guide for health professionals. Edited by Bendich

A and Deckelbaum RJ. New Jersey: Humana Press Inc., NJ, 1997;135-152.
23. Weisburger JH. Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants, and carotenoids. Am J Clin Nutr 1991;53:226S-37S.

BEHAVIOR

Paige Green-McDonald, Ph.D., Assistant professor of Medicine, Division of Epidemiology and Biostatistics, Howard University Cancer Center.

Educational Objectives

Upon successful completion of this component, participants will be able to:

1. Describe the role of behavioral research in the prevention, early detection, and control of cancer
2. Identify and discuss priorities in behavioral research related to cancer prevention and control
3. Identify and describe health behavior theories in cancer prevention and control
4. Critically discuss and evaluate behavioral interventions used in cancer 0provention and control

Instructional Units/Topics

1. Risk factors: tobacco use, diet, physical exercise, alcohol use, stress, racism, socioeconomic status
2. Priorities in behavioral research in cancer prevention and control
3. Individual health behavior theories: Health Belief Model, Theory of Reasoned Action, Transtheoretical model of change, Prospect Theory, Transactional Model of Stress and Coping
4. Interpersonal health behavior theories: Social Cognitive Theory, social networks and social support, patient-provider communication
5. PRECEDE-PROCEED Planning Model
6. Race in cancer prevention and control
7. Behavioral intervention studies

References:

GRADUATE ONCOLOGY COURSE
SYLLABUS

EPIDEMIOLOGY OF CANCER

Lucile Adams-Campbell, Ph.D., Director HUCC & Professor of Medicine
Flora Ukoli, MD, D.P.H., MPH, Pamela Carter-Nolan, Ph.D., Division of Epidemiology and Biostatistics, HUCC

Educational Objectives:

1. To provide an understanding of epidemiology in cancer research.
2. To provide an understanding of the epidemiology of specific cancers which effect minority communities (e.g., breast, prostate, GI, etc.).
3. To evaluate the cause-effect relationships that may exist between risk factors and specific cancers effecting minority populations (e.g., evaluating the consistency of epidemiologic data with etiologic hypotheses identified either clinically or experimentally)..
4. To provide the basis for developing and understanding preventive procedures and public health practice.

Instructional Units/Topics:

1. *Basic Concepts*
   - Causation and Causal Inference
   - Molecular Epidemiology in Cancer Prevention
2. *Magnitude of Cancer*
   - Cancer Incidence, Mortality, and Survival among Racial and Ethnic Minority Groups in the US
3. *Causes of Cancer*
   - Risk factors associated with cancers that effect Minority Populations
4. *Cancer Prevention and Control*
   - Principles and Applications of Cancer Prevention
   - Health Education and Health Promotion
   - Clinical Trials
   - Fundamental Issues in Screening

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1. American Cancer Society. 1998. Cancer Statistics -- 1998. CA Cancer J Clin. 48(1).
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16. Renton A. 1994. Epidemiology and causation: a realist view. J Epidemiol Community Health 48:79-85.
17. Rothman KJ. 1976. Causes. Am J Epidemiol 104:587-592.

CANCER STATISTICS AND STATISTICAL METHODS; EPIDEMIOLOGY OF CANCER

Kyung Sook Kim, Ph.D., assistant professor, Division of Epidemiology and Biostatistics, HUCC

Introduction to Biostatistics

Kyung Sook Kim, Ph.D., Assistant professor, Department of Community Health and Family Practice.

Educational Objectives:

Upon successful completion of this component, participants will be able to

1. Develop an understanding of the statistical concepts and of how and when to apply various statistical techniques.
2. Better understand published medical literature and critically evaluate authors' conclusions.

Instructional Units:

The student shall distinguish the following concepts:

1. Descriptive statistics/ Graphic representation
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6. Survival Curve
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2. Johnson, R., Elementary Statistics, 4th ed., North Scituate, Mass: Duxbury Press.
   Milton, S., Statistical Methods in the Biological and Health Sciences, 3rd ed., WCB/McGraw-Hill.
3. Essex-Sorlie, D., Medical Biostatistics & Epidemiology, APPLETON & LANGE, 1995.
4. Lee, E., Statistical Methods for Survival Data Analysis, 2nd ed., John Wiley & Sons, Inc., 1992.

CELL CYCLE AND CANCER

Hassan Ashktorab, Ph.D., *graduate assistant professor*, GI Division, Department of Medicine, Howard University; Gastrointestinal Carcinogenesis Laboratory, Howard University Cancer Center.

Instructional Units/Topics

1. Biochemistry and genetics of the cell cycle; growth factors, growth factor receptors, and receptor-mediated signaling; cell cycle regulators (cyclins and cyclin-dependent kinases (CDKs); inhibitors of CDKs (CDIs)
2. Cell cycle-related gene expression, checkpoints, transitions.
3. Cell cycle dysregulation and oncogenesis
4. p53, Rb, etc tumor suppressors and regulation of cell cycle

MOLECULAR DIAGNOSIS OF CANCER

Hassan Ashktorab, Ph.D., *graduate assistant professor*, GI Division, Department of Medicine, Howard University; Gastrointestinal Carcinogenesis Laboratory, Howard University Cancer Center.

Instructional Units/Topics

1. Current methods for molecular diagnosis of various cancers
2. Molecular basis of diagnostic techniques for breast cancer
3. Translational studies and clinical benefits

References:

Cell Cycle and Cancer

1. Bates S; Peters G Cyclin D1 as a cellular proto-oncogene. Semin Cancer Biol 1995 Apr;6(2):73-82
2. Biggs JR; Kraft AS. Inhibitors of cyclin-dependent kinase and cancer. J Mol Med. 1995 Oct;73(10):509-14
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9. Prall OW, Rogan EM, Musgrove EA, Watts CK, Sutherland RL c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry.Mol Cell Biol 1998 Aug;18(8):4499-508
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12. Weinberg RA. How cancer arises. Sci Am 1996 Sep;275(3):62-70

Cell cycle machinery

1. Charollais RH; Tiwari S; Thomas. Into and out of G1: the control of cell proliferation. Biochimie 1994;76(9):887-94
2. Deshaies RJ. The self-destructive personality of a cell cycle in transition. Curr Opin Cell Biol 1995 Dec;7(6):781-9
3. Grana X; Reddy EP. Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases

(CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). Oncogene 1995 Jul 20;11(2):211-9.
4. Heichman KA; Roberts JM. CDC16 controls initiation at chromosome replication origins. Mol Cell 1998 Feb;1(3):457-63
5. Heichman KA; Roberts JM.Rules to replicate by Cell 1994 Nov 18;79(4):557-62
6. Kranenburg O; van der Eb AJ; Zantema A Cyclin-dependent kinases and pRb: regulators of the proliferation- differentiation switch. FEBS Lett 1995 Jun 26;367(2):103-6
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8. Nurse P; Masui Y; Hartwell L. Understanding the cell cycle. Nat Med 1998 Oct;4(10):1103-6
9. Peeper DS; van der Eb AJ; Zantema A The G1/S cell-cycle checkpoint in eukaryotic cells. Biochim Biophys Acta 1994 Dec 30;1198(2-3):215-30
10. Peeper DS; Bernards R. Communication between the extracellular environment, cytoplasmic signalling cascades and the nuclear cell-cycle machinery. FEBS Lett 1997 Jun 23;410(1):11-6
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15. Sgambato A; Flamini G; Cittadini A; Weinstein IB. Tumori Abnormalities in cell cycle control in cancer and their clinical implications. 1998 Jul-Aug;84(4):421-33
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18. Zhou JR; Mukherjee P; Gugger ET; Tanaka T; Blackburn GL; Clinton. Inhibition of murine bladder tumorigenesis by soy isoflavones via alterations in the cell cycle, apoptosis, and angiogenesis. Cancer Res 1998 Nov 15;58(22):5231-8

NUTRITION AND CANCER

Tanya Agurs-Collins, Ph.D., R.D., Nutrition Epidemiologist, Howard University Cancer Center, *and* *Assistant Professor*, Department of Community Health and Family Practice, Howard University College of Medicine.

Educational Objectives:
Upon successful completion of this component, participants will be able to:

Describe the biological principles of nutritional oncology
Understand the mechanisms/pathways that link nutritional status and the etiology of cancer

Understand the relationship between diet, nutrition and cancer prevention.

Instructional Units/Topics:

1. Fundamentals of nutrition: applications to cancer research
2. Epidemiology basis of nutritional influences on cancer
3. Dietary assessment and cancer prevention
4. Fruits and vegetable intake and cancer prevention
5. Energy balance, anthropometry and cancer
6. Dietary fiber, carbohydrate and cancer
7. Dietary lipid, alcohol and cancer
8. Functional foods and cancer prevention

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1. Nutritional Oncology, Edited by: Heber D, Blackburn G, Go VLW, Harcourt Brace & Company 1998.
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8. Skog KI, Johansson MA, Jagerstad MI. Carcinogenic heterocyclic amines in model systems and cook foods: a review on formation, occurrence and intake. Food Chem Toxicol 1998 Sept-oct;36(9-10):879-96.
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CANCER CHEMOTHERAPY: AGENTS AND TARGETS

Fred Lombardo, Pharm. D., College of Pharmacy and Pharmacal Sciences, and H.U. Cancer Center Faculty.

Topics/instructional units
Agents utilized in cancer therapy
Cell cycle specificity: cytotoxicity

S-phase (antimetabolites)
1. Antifolates (Methotrexate, Trimetrexate)
2. Antipyrimidines (Cytarabine, 5-Fluorouracil, etc)
3. Antipurines (6-Mercaptopurine, 6-Thioguanine)
4. Miscellaneous Agents (Hydroxyurea, Procarbazine)

G-2 Phase
Bleomycin

M-Phase
1. Vinca alkaloids (Vincristine, Vinblastine, Vinorelbine)
2. Podophylotoxins (Etoposide, Teniposide)
3. Taxanes (Taxol, Taxotere)

G-0 Phase
1. Nitrosoureas
2. Alkylators
3. Intercalators

G-1 Phase
1. L-asparaginase
2. Steroids

Cell cycle Non-Specificity
Apoptosis
Anti-Angiogenic Agents
Monoclonal Antibodies in cancer therapy
Cytokines/Chemokines

APOPTOSIS AND CANCER

Theodore A. Bremner, Ph.D., *graduate associate professor*, Department of Biology, and Tumor Biology Laboratory, Howard University Cancer Center; adjunct associate professor of molecular biology (research), Department of Cell Biology, Molecular Biology, and Biochemistry, Brown University.

Educational Objectives:

Upon successful completion of this component, participants will be able to

1. Describe apoptotic pathways and discuss the importance of apoptosis as a mechanism of tissue homeostasis, and its abrogation in cancer.
2. Discuss the role of the Bcl-2 family of proteins in modulating apoptosis.
3. Understand the loss of susceptibility to apoptosis as a mechanism of tumor promotion, and resistance to cancer chemotherapeutic drugs.
4. Describe the caspase activation cascade involved in Fas-mediated apoptosis; the mechanism of activation of caspases, and the cleavage of critical death substrates during programmed cell death.
5. Describe the role of the p53 tumor-suppressor gene in the induction of apoptosis in response to DNA damage or viral infection.
6. Describe the association between the p53 proline/arginine 72 polymorphism and risk of human papillomavirus-associated cervical cancer.
7. Discuss the mechanisms of action of oncolytic viruses on p53-negative tumors.
8. Describe laboratory methods of detection and quantitation of apoptosis.

Instructional Units:

1. Tissue homeostasis: proliferation and apoptosis. Survival/anti-apoptotic signaling: Akt/PKB and NF-κB in anti-apoptotic signaling.
2. Oncogenes and tumor suppressor genes that affect (primarily) cell cycling, apoptosis, or both (excluding *ras*, *p53*, and *Rb*, covered earlier): *fos*, *APC*, *DCC*, *PTEN*, *Cbl*, *MTS1*, *BRCA1*. Fos and gene silencing via methylation; the APC/β-catenin signaling pathway; PTEN and abrogation of survival signaling; DCC, a dependence receptor. Fearon-Vogelstein model of colorectal carcinogenesis.
2. Receptor-mediated apoptotic signaling: Fas/Apo-1/CD95 and Fas ligand (FasL) in immune regulation and cell-mediated killing.
3. Proapoptotic and antiapoptotic molecules: Bcl-2 family members, NF-κB, cIAP, superoxide.
4. Mechanisms of p53 potentiation of apoptosis in response to DNA damage and viral infection: ATM kinase and phosphorylation of p53; trancriptional regulation of *bax* and *bcl-2*; Fas trafficking.
5. Viral suppression of apoptosis: FLIPs, SV40 LTA, HPV-16 and HPV-18 E6 proteins and functional inactivation of p53.
6. Laboratory methods for the detection and quantitation of apoptosis: DNA laddering, nuclear morphology, phosphatidyl serine (PS) externalization and Annexin V binding; TUNEL analysis, flow cytometry (FCM) using propidium iodide, Hoechst 33342, Annexin V-FITC labeling. Interpretation of FCM data.

WWW RESOURCES

American Cancer Society: http://www.cancer.org/
Apoptosis Online: http://www.apopnet.com/
BioMedNet: http://www.BioMedNet.com
BioMedLink: http://biomedlink.com
Cancer Coalition: http://www.cancercoalition.org
Cell and Molecular Biology Online: http://www.cellbio.com/
Cells Alive: http://www.cellsalive.com
Howard Hughes Medical Institute: http://www.hhmi.org
Leukemia Society of America: http://www.leukemia.org/docs/fs_leuk_rel.html
National Library of Medicine (Internet Grateful Med; Pub Med; etc. Literature searches in all areas of the biomedical sciences): http://www.nlm.nih.gov/
National Cancer Institute (NCI): Cancer Trials; CancerNet™, NCI Event Calendar; Research at NCI; Office of International Affairs, etc.: http://www.nci.nih.gov

OncoLink (University of Pennsylvania): http://oncolink.upenn.edu/upcc/
UICC (International Union Against Cancer/Union Internationale Contre le Cancer): http://www.uicc.org

Tutorials
Flow cytometry in cell cycle analysis and apoptosis: An excellent tutorial is available at the Derek Davies Home Page (Imperial Cancer Research Fund (ICRF) , London:

http://www.lif.icnet.uk/axp/facs/davies/annexin2.gif
Can be accessed through BioMedLink: http://biomedlink.com

About Apoptosis, by P. Henkart, NIH
A Brief Introduction to Apoptosis by L. W. Browder
Apoptosis, Radiosensitivity and the Cell Cycle, by W Gillies McKenna, M.D., Ph.D. in OncoLink
Can be accessed through http://www.apopnet.com/

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BIOLOGY OF RADIOTHERAPY

Raj Sridhar, Ph.D.,graduate associate professor, Dept. of Radiation Oncology, Howard University Hospital.

Topics/Instructional Units

1. Types of radiation used in cancer therapy
2. Interactions of radiation with matter
3. Concept of radiation
4. Dose-response curves, cellular target for radiation damage
5. Repair of radiation damage
6. Fractionation of radiation dose
7. Normal tissue tolerance and radiation response of tumors
8. Goals of radiation therapy and multi-modality treatment of cancer
9. Radiosensitization, radiopprotection and drug radiation interactions
10. Brachytherapy

References:

CHEMOPREVENTION AND CARCINOGENESIS

Joel Schwartz, D.M.D., D.M.Sc., Director of Research, College of Dentistry, Howard University

Department of Oral Maxillofacial Pathology, Rm. 2A-3, Laboratory, 2F-8. Pjones: (202) 806-0094; (202) 806-0345

Educational Objectives

Upon successful completion of this component, participants will have knowledge of:

1. The possible mechanisms for the prevention of cancer.
2. The interaction of nutrients with cellular processes.
3. The specific oxidative-redox features of nutrients that result in the modification of cellular processes.
4. The redox characteristics of tumor suppressor genes.
5. The redox features [describing features] of programmed cell death and DNA repair. (?)
6. Transcription response to redox molecules.
7. Alterations in the cell cycle as derivatives of chemopreventive actions.
8. Carcinogenesis described by molecular and histopathologic markers.
9. Molecular and histopathologic markers of chemopreventive function.

Topics/Instructional Units

1. Programmed cell death: the tumor suppressor and immune generated pathways: assays to assess chemopreventive modifications.
2. Laboratory methods to detect nutrient chemopreventive agents: animal and laboratory assays.
3. Laboratory methods to detect novel mutations in tumor suppressor genes and protocols to determine novel for early malignant transformation.
4. Focus on the interaction between nutrient chemopreventives, and/or diet and the process of oral carcinogenesis.

References:

CANCER GENETICS AND GENOMICS

Carolyn Whitfield Broome, Ph.D., Associate professor, Department of Biochemistry and Molecular Biology, College of Medicine, Howard University, and
Rick Kittles, Ph.D., Human Genome Research Associate, Cancer center, Howard University.

Educational Objectives

Instructional Units/Topics

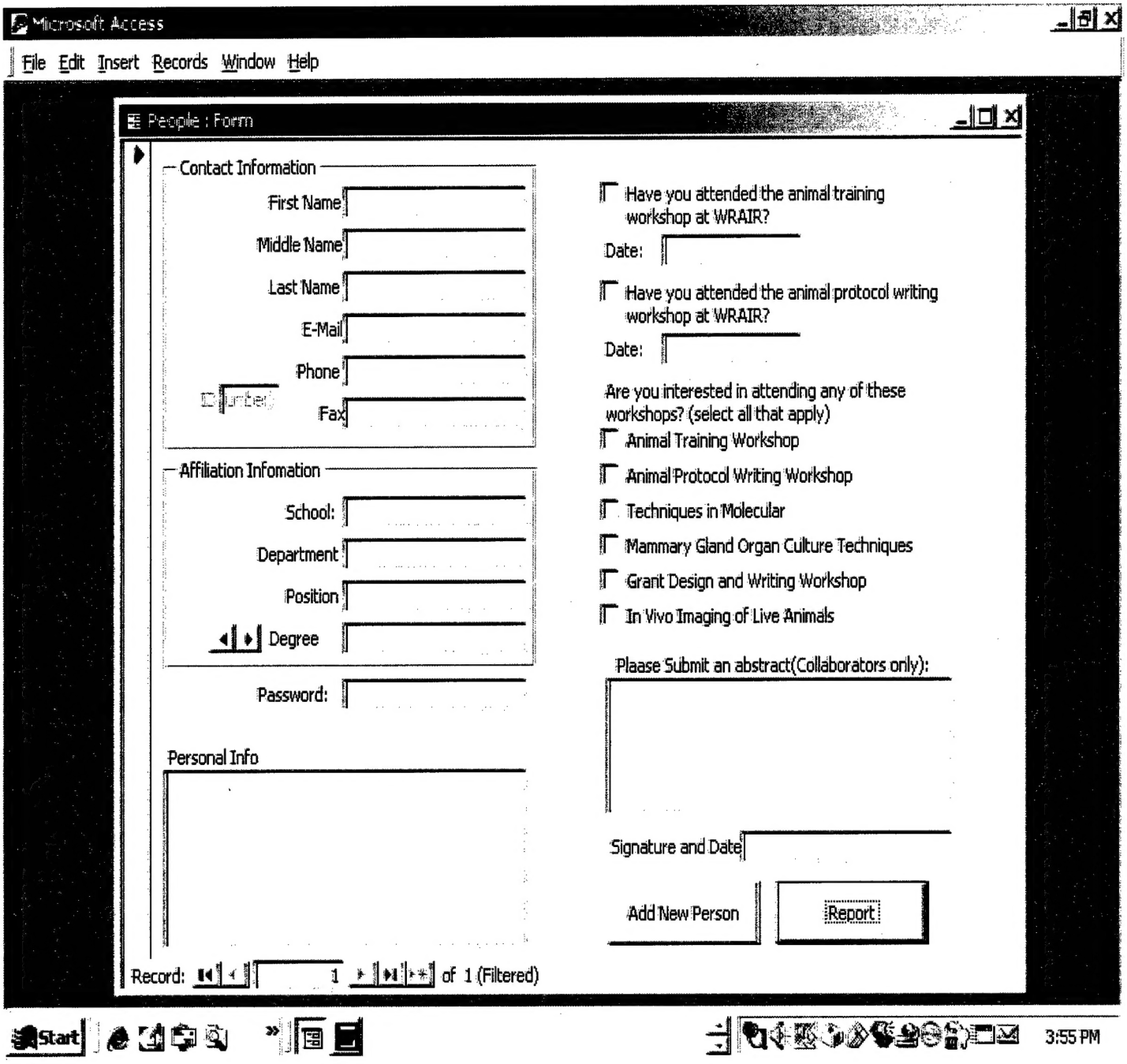
References:

HOWARD/HOPKINS PARTNERSHIP
PILOT PROJECT INITIATIVE

PI: Agnes Day

ABSTRACT
(DO NOT EXCEED 200 WORDS)

The alteration of normal cells to benign tumors, to malignancies and subsequent metastasis involves a highly complex series of events. Proteolytic degradation is the currently accepted method by which metastatic cells abrogate the basement membrane and conncetive tissue matrix to gain access to the circulatory and lymphatic systems for dispersal. The goal of this study is to ascertain whether differential regulation occurs in genes encoding proteins of the basement membrane and extracellular matrix, and how this event contributes to the metastatic phenotype. Previous studies using slot blot and RT-PCR analyses of breast and colon cell lines have demonstrated altered transcriptional levels of decorin, type I collagen and osteonectin within (solid tumors-vs- ascites) and between cell types (breast-vs-colon). Since cells in culture may undergo regulatory events that alter the expression of matrix proteins, the next phase of this study must examine non-cultured clinical samples. Towards this end, DNA, RNA and protein will be isolated from clinical samples from African American, Caucasian and Korean women. RNA will be amplified and used in gene microarray analyses. Purified proteins from each sample will be assayed on 2-D protein array electrophoresis. DNA will be analyzed by Southern blot studies. Promoter regions of selected genes will be isolated and utilized in gel retardation studies for determination of regulatory mutations. This multivariate approach to the study of breast cancer may yield a molecular profile of the metastatic phenotype, and whether this phenotype is genetically similar in different ethnic/racial groups.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Are you interested in attending any of these workshops? (select all that apply)
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Mammary Gland Organ Culture Techniques
Grant Design and Writing Workshop
In Vivo Imaging of Live Animals
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